

# **KnowItAll Software Training**

---

## Mass Spectrometry Search

# Mass Spectrometry Spectral Searching

## How to Perform a Mass Spectrometry Spectral Search

### Purpose

This exercise demonstrates how to perform a Mass Spectrometry spectral search.

### Objectives

This exercise will teach you:

- How to perform a straightforward search
- How to perform a reverse search
- How to complete a mixture analysis
- How to perform an Adaptive search (similar compound search)
- How to perform simultaneous multiple MS spectra search

### Background

Spectral searching against reference databases is frequently used in the analysis of unknown compounds. KnowItAll has full-featured MS spectrum comparison tools for this purpose.

#### *Training Files Used in This Lesson*

In C:\Users\Public\Documents\Wiley\KnowItAll\Samples folder

- \MS\1,1,1-Trichlorobutane - Adaptive Search demo
- \MS\2-Hydroxybenzoic acid
- \Mixture Analysis\MS Examples\MS Mixture of Two 1
- \Mixture Analysis\MS Examples\MS Mixture of Two 2
- \Mixture Analysis\MS Examples\MS Mixture of Three
- \GC-MS\Barbiturate GC-MS.d
- \Mixture Analysis\MS Examples\Components.SDBX

#### *KnowItAll Applications Used*

- SearchIt™
- Minelt™

## Algorithms

### Research article

Journal of  
MASS  
SPECTROMETRY

Received: 5 October 2014

Revised: 16 February 2015

Accepted: 5 March 2015

Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jms.3591

# Evaluation of mass spectral library search algorithms implemented in commercial software

Andrey Samokhin,<sup>a\*</sup> Ksenia Sotnezova,<sup>a</sup> Vitaly Lashin<sup>b</sup> and Igor Revelsky<sup>a</sup>

MS SEARCH

Composite algorithm

$$SI = \frac{N_U \cdot \left[ \frac{\left( \sum W_L \cdot W_U \right)^2}{\sum W_L^2 \cdot \sum W_U^2} \right] + \left[ \sum \left( \frac{R_U}{R_L} \right)^n \right]}{N_U + N_{U\&L}}$$

Spectrum search type – identity (normal)

Presearch – default

Included Libs – MainLib

Apply limits – unchecked

Use constraints – unchecked

Dot-product algorithm

$$SI = \frac{\left( \sum W_L \cdot W_U \right)^2}{\sum W_L^2 \cdot \sum W_U^2}$$

Spectrum search type – similarity (simple)

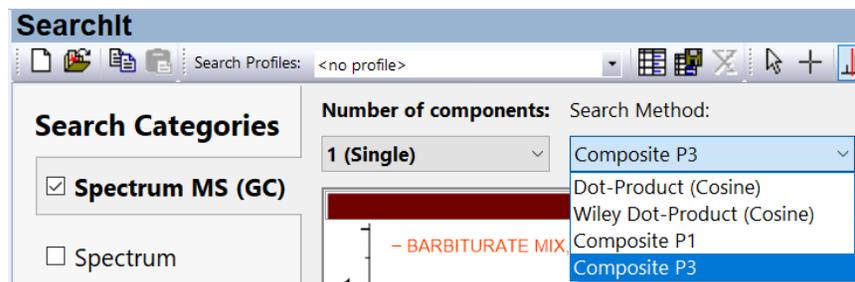
Presearch – default

Included Libs – MainLib

Apply limits – unchecked

Use constraints – unchecked

A. Samokhin, K. Sotnezova, V. Lashin, I. Revelsky. Evaluation of mass spectral library search algorithms implemented in commercial software. *Journal of Mass Spectrometry*. 2015, 50, 820-825.



#### Search Methods:

- Dot-Product (Cosine): second equation in above graph
- Wiley Dot-Product (Cosine): the Finnigan algorithm that verified at least 12 of the largest 16 peaks and the base peak match before continuing with the dot-product calculation
- Composite P1: first equation in above graph
- Composite P3: first equation in above graph  
P1 and P3 are different by the power applied to the weighted intensity of peak.

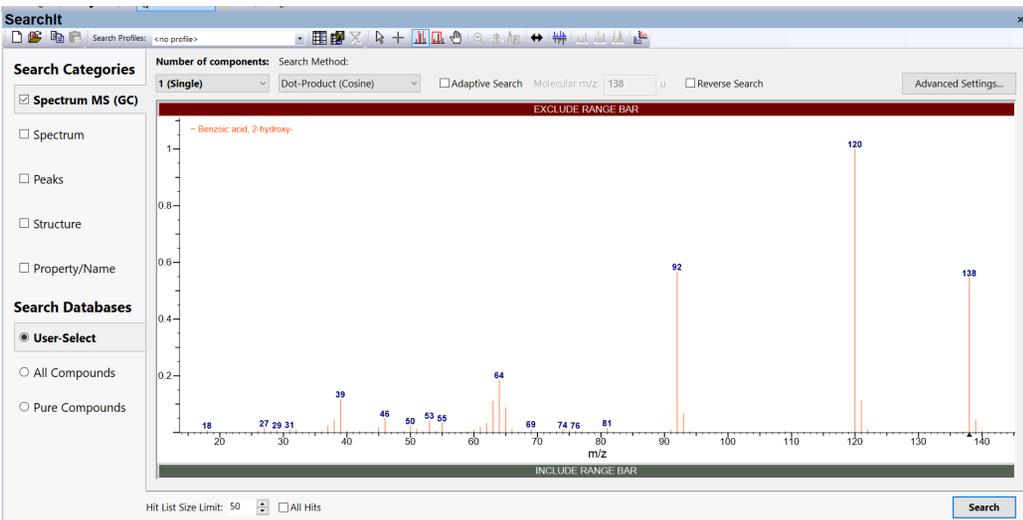
#### Optimized Corrections:

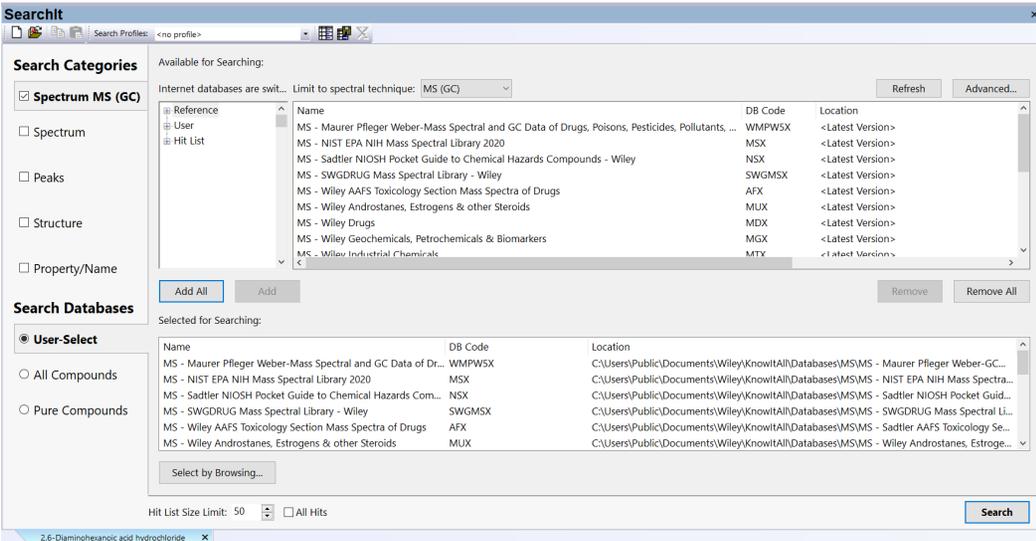
- Mass Defection is the difference between a compound's exact mass and its nominal mass. It is automatically applied for a MS search. Examples:
  - For hydrocarbon compounds, when  $m/z$  value is over 500, use 0.99888.
  - For polybrominated compounds, when there are more than 5 Br atoms and  $m/z$  value is over 800, use 1.00087.
- Spectral Skewing is caused by analyte's concentration changing during scan. A linear compensation factor (positive for ascending or negative for descending slope) is calculated for each search match. This factor corrects the intensities as follows:

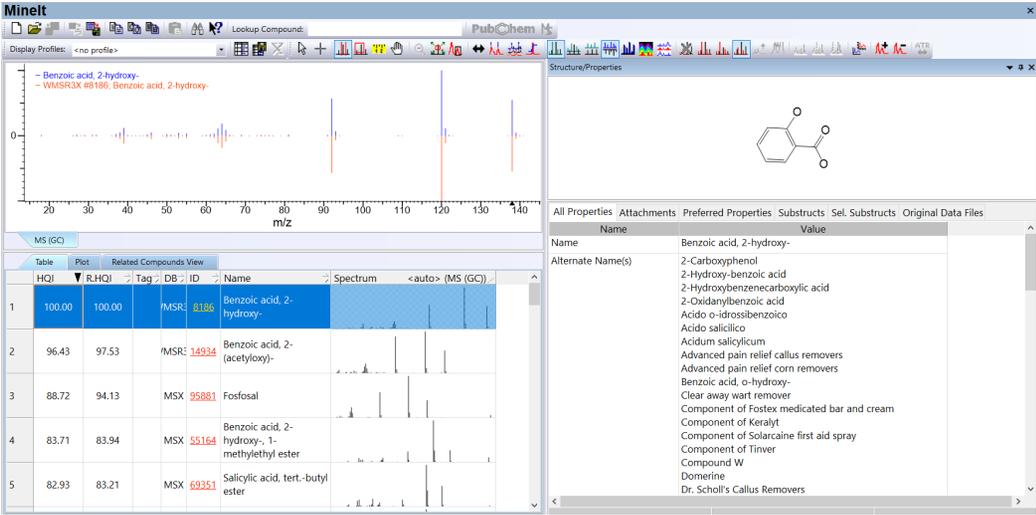
$$I(\text{corrected}) = I * f * m,$$

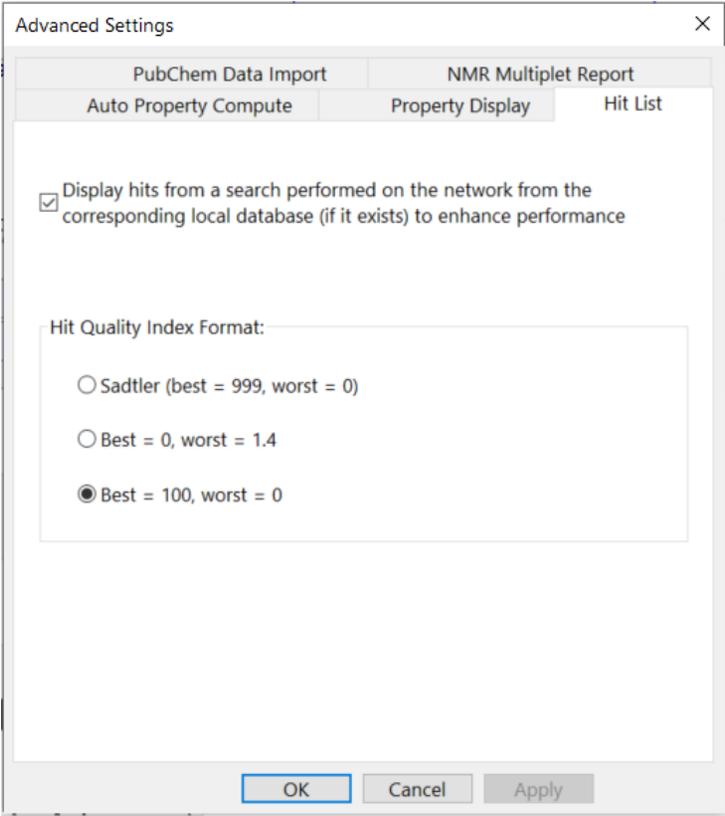
where I=intensity, f=OC factor, and  $m=m/z$  value

## Straightforward Search

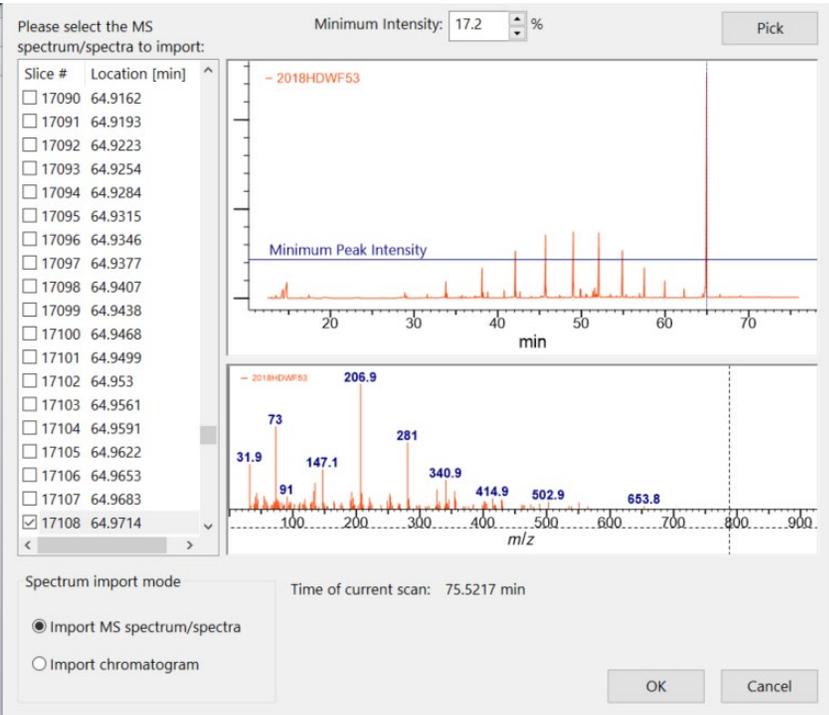
	Action	Result
1	<p>Open the KnowItAll <b>SearchIt</b> application by clicking its icon in the KnowItAll application.</p> <p>Check <b>Spectrum</b> and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS folder</p> <p>Select the file <b>2-Hydroxybenzoic acid</b>.</p> <p>Click <b>Open</b>.</p> <p>Ensure <b>Adaptive Search</b> or <b>Reverse Search</b> is unchecked.</p>	 <p>The screenshot shows the SearchIt application window. The 'Search Categories' panel on the left has 'Spectrum MS (GC)' checked. The 'Search Databases' panel has 'User-Select' selected. The main area displays a mass spectrum for 'Benzoic acid, 2-hydroxy-'. The x-axis is labeled 'm/z' and ranges from 20 to 140. The y-axis represents relative intensity from 0 to 1.0. The base peak is at m/z 120. Other significant peaks are at m/z 92 and 138. A red bar at the top of the plot area is labeled 'EXCLUDE RANGE BAR' and a black bar at the bottom is labeled 'INCLUDE RANGE BAR'. The 'Number of components' is 1 (Single) and the search method is 'Dot-Product (Cosine)'. The molecular weight is listed as 138 u. A 'Search' button is located at the bottom right of the plot area.</p>

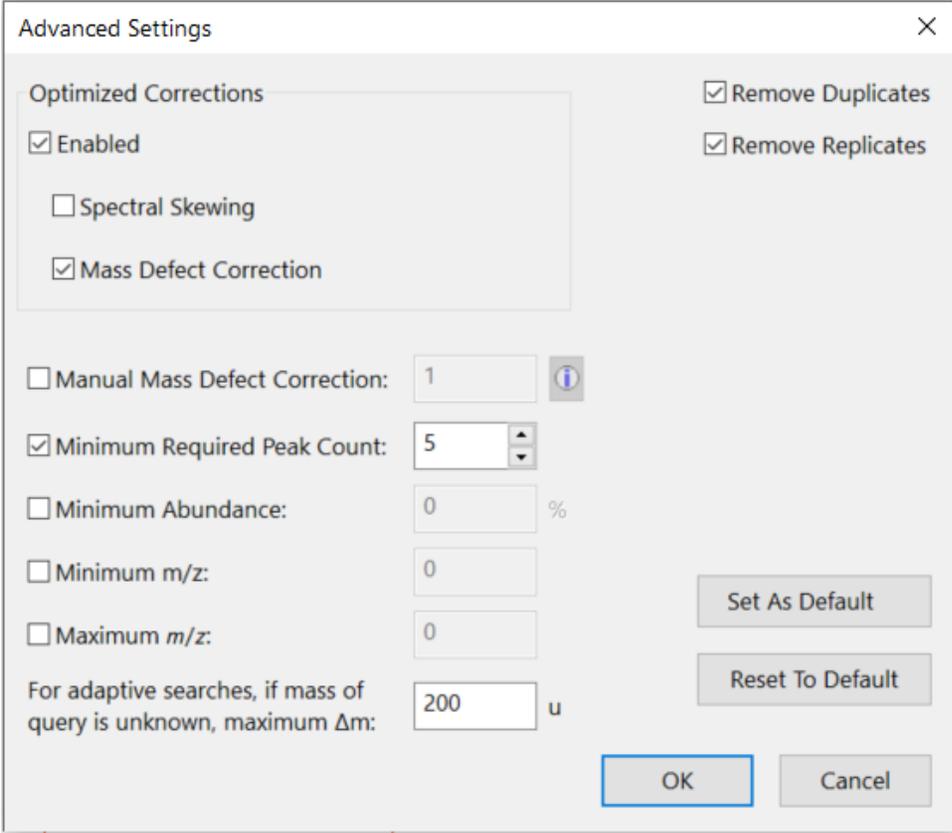
Action	Result																																																																								
<p>2 Click <b>User-Select</b>.</p> <p>Use <b>Remove All</b> to clean selected databases.</p> <p>Use <b>Add All</b> to add all MS (GC) databases.</p> <p>Click <b>Search</b>.</p>	 <p>The screenshot shows the SearchIt application window. On the left, under 'Search Categories', 'Spectrum MS (GC)' is selected. Under 'Search Databases', 'User-Select' is selected. The main area displays a table of available internet databases for searching, with columns for Name, DB Code, and Location. A table of 'Selected for Searching' databases is also visible below.</p> <table border="1"> <thead> <tr> <th>Internet databases are swit...</th> <th>Limit to spectral technique: MS (GC)</th> <th>Refresh</th> <th>Advanced...</th> </tr> </thead> <tbody> <tr> <td>Reference</td> <td>Name</td> <td>DB Code</td> <td>Location</td> </tr> <tr> <td>User</td> <td>MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, ...</td> <td>WMPWSX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>Hit List</td> <td>MS - NIST EPA NIH Mass Spectral Library 2020</td> <td>MSX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td> <td>NSX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - SWGDRUG Mass Spectral Library - Wiley</td> <td>SWGMSX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs</td> <td>AFX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - Wiley Androstanes, Estrogens &amp; other Steroids</td> <td>MUX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - Wiley Drugs</td> <td>MDX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - Wiley Geochemicals, Petrochemicals &amp; Biomarkers</td> <td>MGX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td></td> <td>MS - Wiley Industrial Chemicals</td> <td>MTX</td> <td>&lt;Latest Version&gt;</td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Selected for Searching:</th> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td></td> <td>MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Dr...</td> <td>WMPWSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Maurer Pfleger Weber-GC...</td> </tr> <tr> <td></td> <td>MS - NIST EPA NIH Mass Spectral Library 2020</td> <td>MSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - NIST EPA NIH Mass Spectra...</td> </tr> <tr> <td></td> <td>MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Com...</td> <td>NSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler NIOSH Pocket Guid...</td> </tr> <tr> <td></td> <td>MS - SWGDRUG Mass Spectral Library - Wiley</td> <td>SWGMSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - SWGDRUG Mass Spectral LI...</td> </tr> <tr> <td></td> <td>MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs</td> <td>AFX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler AAFS Toxicology Se...</td> </tr> <tr> <td></td> <td>MS - Wiley Androstanes, Estrogens &amp; other Steroids</td> <td>MUX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Androstanes, Estroge...</td> </tr> </tbody> </table>	Internet databases are swit...	Limit to spectral technique: MS (GC)	Refresh	Advanced...	Reference	Name	DB Code	Location	User	MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, ...	WMPWSX	<Latest Version>	Hit List	MS - NIST EPA NIH Mass Spectral Library 2020	MSX	<Latest Version>		MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NSX	<Latest Version>		MS - SWGDRUG Mass Spectral Library - Wiley	SWGMSX	<Latest Version>		MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs	AFX	<Latest Version>		MS - Wiley Androstanes, Estrogens & other Steroids	MUX	<Latest Version>		MS - Wiley Drugs	MDX	<Latest Version>		MS - Wiley Geochemicals, Petrochemicals & Biomarkers	MGX	<Latest Version>		MS - Wiley Industrial Chemicals	MTX	<Latest Version>	Selected for Searching:	Name	DB Code	Location		MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Dr...	WMPWSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Maurer Pfleger Weber-GC...		MS - NIST EPA NIH Mass Spectral Library 2020	MSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - NIST EPA NIH Mass Spectra...		MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Com...	NSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler NIOSH Pocket Guid...		MS - SWGDRUG Mass Spectral Library - Wiley	SWGMSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - SWGDRUG Mass Spectral LI...		MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs	AFX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler AAFS Toxicology Se...		MS - Wiley Androstanes, Estrogens & other Steroids	MUX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Androstanes, Estroge...
Internet databases are swit...	Limit to spectral technique: MS (GC)	Refresh	Advanced...																																																																						
Reference	Name	DB Code	Location																																																																						
User	MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, ...	WMPWSX	<Latest Version>																																																																						
Hit List	MS - NIST EPA NIH Mass Spectral Library 2020	MSX	<Latest Version>																																																																						
	MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NSX	<Latest Version>																																																																						
	MS - SWGDRUG Mass Spectral Library - Wiley	SWGMSX	<Latest Version>																																																																						
	MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs	AFX	<Latest Version>																																																																						
	MS - Wiley Androstanes, Estrogens & other Steroids	MUX	<Latest Version>																																																																						
	MS - Wiley Drugs	MDX	<Latest Version>																																																																						
	MS - Wiley Geochemicals, Petrochemicals & Biomarkers	MGX	<Latest Version>																																																																						
	MS - Wiley Industrial Chemicals	MTX	<Latest Version>																																																																						
Selected for Searching:	Name	DB Code	Location																																																																						
	MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Dr...	WMPWSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Maurer Pfleger Weber-GC...																																																																						
	MS - NIST EPA NIH Mass Spectral Library 2020	MSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - NIST EPA NIH Mass Spectra...																																																																						
	MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Com...	NSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler NIOSH Pocket Guid...																																																																						
	MS - SWGDRUG Mass Spectral Library - Wiley	SWGMSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - SWGDRUG Mass Spectral LI...																																																																						
	MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs	AFX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler AAFS Toxicology Se...																																																																						
	MS - Wiley Androstanes, Estrogens & other Steroids	MUX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Androstanes, Estroge...																																																																						

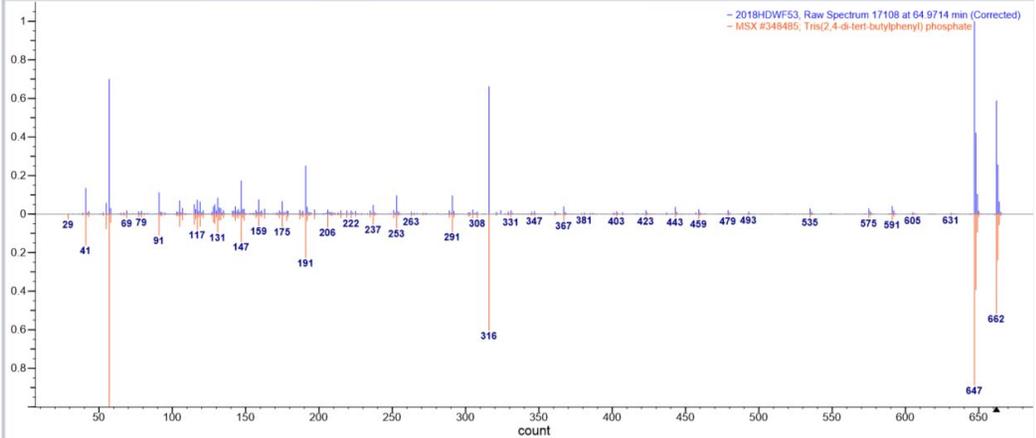
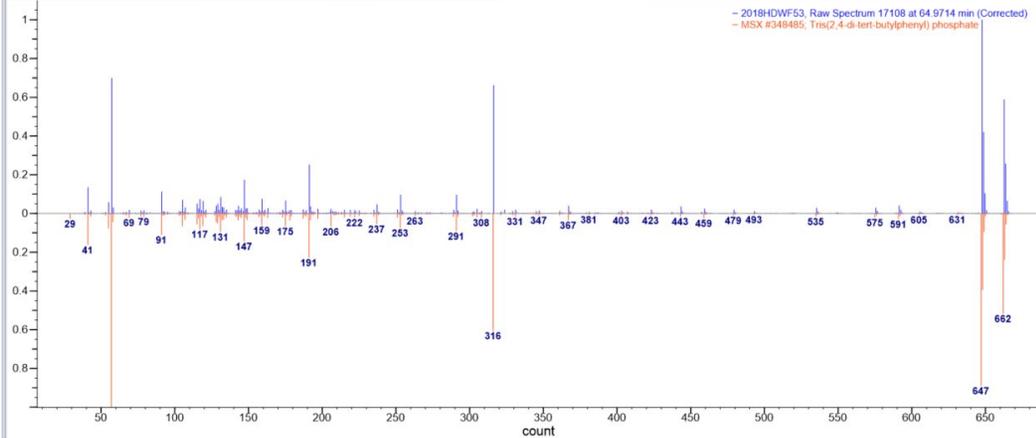
	Action	Result																																										
3	<p>Click the <b>Butterfly view</b> icon  to place the unknown and reference spectrum in the opposite Y-direction.</p>	<p>A target from a database is found.</p>  <p>Hits are initially sorted by the <b>Hit Quality Index (HQI)</b>. <b>Reverse Hit Quality Index (R.HQI)</b> is also calculated for each reference spectrum.</p> <table border="1" data-bbox="856 641 1396 901"> <thead> <tr> <th>HQI</th> <th>R.HQI</th> <th>Tag</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>100.00</td> <td>100.00</td> <td>MSR</td> <td>8186</td> <td></td> <td>Benzoic acid, 2-hydroxy-</td> <td></td> </tr> <tr> <td>96.43</td> <td>97.53</td> <td>MSR</td> <td>14934</td> <td></td> <td>Benzoic acid, 2-(acetyloxy)-</td> <td></td> </tr> <tr> <td>88.72</td> <td>94.13</td> <td>MSX</td> <td>95881</td> <td></td> <td>Fosfosal</td> <td></td> </tr> <tr> <td>83.71</td> <td>83.94</td> <td>MSX</td> <td>55164</td> <td></td> <td>Benzoic acid, 2-hydroxy-, 1-methylethyl ester</td> <td></td> </tr> <tr> <td>82.93</td> <td>83.21</td> <td>MSX</td> <td>60351</td> <td></td> <td>Salicylic acid, tert-butyl ester</td> <td></td> </tr> </tbody> </table>	HQI	R.HQI	Tag	DB	ID	Name	Spectrum	100.00	100.00	MSR	8186		Benzoic acid, 2-hydroxy-		96.43	97.53	MSR	14934		Benzoic acid, 2-(acetyloxy)-		88.72	94.13	MSX	95881		Fosfosal		83.71	83.94	MSX	55164		Benzoic acid, 2-hydroxy-, 1-methylethyl ester		82.93	83.21	MSX	60351		Salicylic acid, tert-butyl ester	
HQI	R.HQI	Tag	DB	ID	Name	Spectrum																																						
100.00	100.00	MSR	8186		Benzoic acid, 2-hydroxy-																																							
96.43	97.53	MSR	14934		Benzoic acid, 2-(acetyloxy)-																																							
88.72	94.13	MSX	95881		Fosfosal																																							
83.71	83.94	MSX	55164		Benzoic acid, 2-hydroxy-, 1-methylethyl ester																																							
82.93	83.21	MSX	60351		Salicylic acid, tert-butyl ester																																							

	Action	Result
4	<b>HQI</b> (Hit Quality Index)	<p>The <b>HQI</b> value measures how close the reference spectrum is to that of the query. The default scale of <b>HQI</b> is 0-100. You can change the scale from <b>Minelt &gt; File &gt; Preferences &gt; Hit List</b>.</p>  <p><b>Note: Reverse Search</b> ignores peaks that are in unknown but not in reference. One scenario is that the unknown spectrum might be a mixture and the reference spectrum might be a component.</p>

### Optimized Corrections

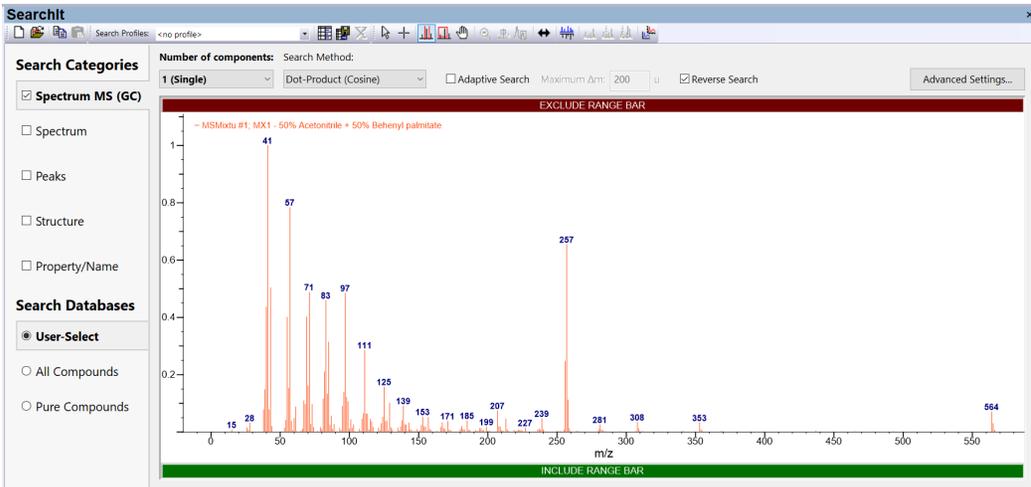
	Action	Result																																								
1	<p>Go back to <b>SearchIt</b>.</p> <p>Click  to start a new search.</p> <p>Click <b>Spectrum</b> and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS</p> <p>Select the file <b>Mass defect correction example</b> Click <b>Open</b>.</p> <p>Click <b>OK</b> (use the default MS 17108)</p>	 <p>Please select the MS spectrum/spectra to import:</p> <table border="1"> <thead> <tr> <th>Slice #</th> <th>Location [min]</th> </tr> </thead> <tbody> <tr><td><input type="checkbox"/></td><td>17090 64.9162</td></tr> <tr><td><input type="checkbox"/></td><td>17091 64.9193</td></tr> <tr><td><input type="checkbox"/></td><td>17092 64.9223</td></tr> <tr><td><input type="checkbox"/></td><td>17093 64.9254</td></tr> <tr><td><input type="checkbox"/></td><td>17094 64.9284</td></tr> <tr><td><input type="checkbox"/></td><td>17095 64.9315</td></tr> <tr><td><input type="checkbox"/></td><td>17096 64.9346</td></tr> <tr><td><input type="checkbox"/></td><td>17097 64.9377</td></tr> <tr><td><input type="checkbox"/></td><td>17098 64.9407</td></tr> <tr><td><input type="checkbox"/></td><td>17099 64.9438</td></tr> <tr><td><input type="checkbox"/></td><td>17100 64.9468</td></tr> <tr><td><input type="checkbox"/></td><td>17101 64.9499</td></tr> <tr><td><input type="checkbox"/></td><td>17102 64.953</td></tr> <tr><td><input type="checkbox"/></td><td>17103 64.9561</td></tr> <tr><td><input type="checkbox"/></td><td>17104 64.9591</td></tr> <tr><td><input type="checkbox"/></td><td>17105 64.9622</td></tr> <tr><td><input type="checkbox"/></td><td>17106 64.9653</td></tr> <tr><td><input type="checkbox"/></td><td>17107 64.9683</td></tr> <tr><td><input checked="" type="checkbox"/></td><td>17108 64.9714</td></tr> </tbody> </table> <p>Spectrum import mode</p> <p><input checked="" type="radio"/> Import MS spectrum/spectra</p> <p><input type="radio"/> Import chromatogram</p> <p>Time of current scan: 75.5217 min</p> <p>Minimum Intensity: 17.2 %</p> <p>Mass Spectrum Peaks (m/z): 73, 91, 147.1, 206.9, 281, 340.9, 414.9, 502.9, 653.8</p>	Slice #	Location [min]	<input type="checkbox"/>	17090 64.9162	<input type="checkbox"/>	17091 64.9193	<input type="checkbox"/>	17092 64.9223	<input type="checkbox"/>	17093 64.9254	<input type="checkbox"/>	17094 64.9284	<input type="checkbox"/>	17095 64.9315	<input type="checkbox"/>	17096 64.9346	<input type="checkbox"/>	17097 64.9377	<input type="checkbox"/>	17098 64.9407	<input type="checkbox"/>	17099 64.9438	<input type="checkbox"/>	17100 64.9468	<input type="checkbox"/>	17101 64.9499	<input type="checkbox"/>	17102 64.953	<input type="checkbox"/>	17103 64.9561	<input type="checkbox"/>	17104 64.9591	<input type="checkbox"/>	17105 64.9622	<input type="checkbox"/>	17106 64.9653	<input type="checkbox"/>	17107 64.9683	<input checked="" type="checkbox"/>	17108 64.9714
Slice #	Location [min]																																									
<input type="checkbox"/>	17090 64.9162																																									
<input type="checkbox"/>	17091 64.9193																																									
<input type="checkbox"/>	17092 64.9223																																									
<input type="checkbox"/>	17093 64.9254																																									
<input type="checkbox"/>	17094 64.9284																																									
<input type="checkbox"/>	17095 64.9315																																									
<input type="checkbox"/>	17096 64.9346																																									
<input type="checkbox"/>	17097 64.9377																																									
<input type="checkbox"/>	17098 64.9407																																									
<input type="checkbox"/>	17099 64.9438																																									
<input type="checkbox"/>	17100 64.9468																																									
<input type="checkbox"/>	17101 64.9499																																									
<input type="checkbox"/>	17102 64.953																																									
<input type="checkbox"/>	17103 64.9561																																									
<input type="checkbox"/>	17104 64.9591																																									
<input type="checkbox"/>	17105 64.9622																																									
<input type="checkbox"/>	17106 64.9653																																									
<input type="checkbox"/>	17107 64.9683																																									
<input checked="" type="checkbox"/>	17108 64.9714																																									

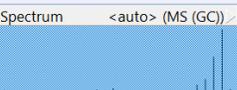
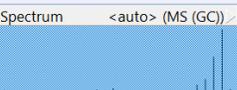
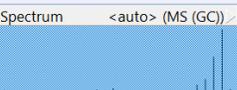
Action	Result
<p>2 Optimized Corrections for MS spectrum is default to be checked.</p> <p><input checked="" type="checkbox"/> Optimized Corrections</p> <p>Click Advanced Setting button</p> <p><input type="button" value="Advanced Settings..."/></p> <p>Click <b>OK</b> to close this dialog</p> <p>Click the <b>Search</b> button</p>	<p>One can see that Mass Defection (the difference between a compound's exact mass and its nominal mass) is automatically applied. Many other criterion, such as Spectral Skewing (analyte's concentration changing during scan), can be applied by users choice to improve a search.</p> 

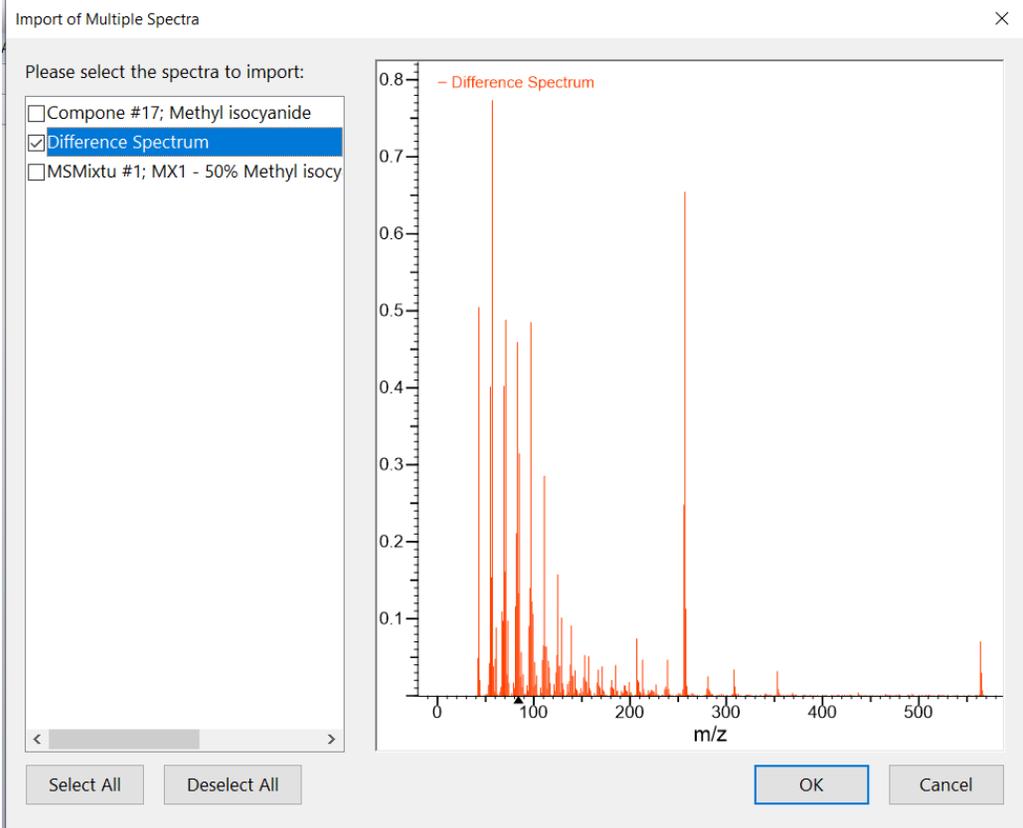
	Action	Result
3		 <p data-bbox="856 802 1650 829">We can see analyte MS and reference MS are perfectly aligned at m/z 662.</p>
4	<p data-bbox="226 852 449 880">Go back to <b>SearchIt</b></p> <p data-bbox="226 917 596 945">Check off <b>Optimized Corrections</b></p> <p data-bbox="226 982 380 1010"><b>Search</b> again</p>	 <p data-bbox="856 1333 1667 1360">At high m/z range, analyte peaks and reference peaks are not aligned nicely.</p>

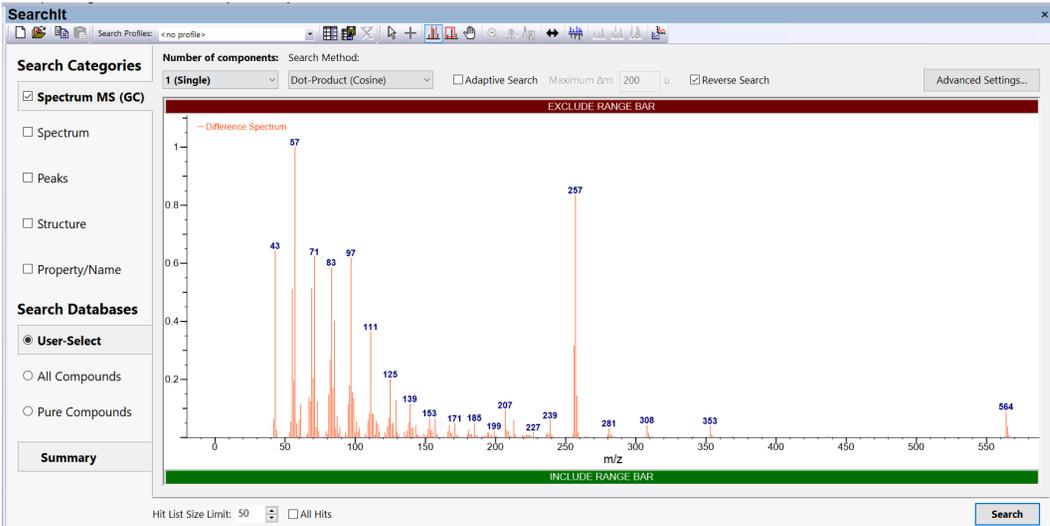
## Reverse Search

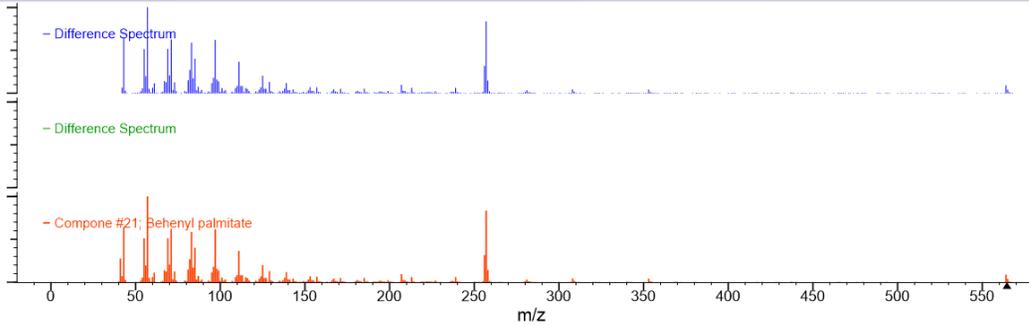
This search ignores peaks that are in unknown but not in reference

	Action	Result
1	<p>Go back to <b>SearchIt</b></p> <p>Click  to start a new search.</p> <p>Click <b>Spectrum</b> and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Mixture Analysis\MS Examples folder</p> <p>Select <b>MS Mixture of Two 1</b> to open</p> <p>Check <b>Reverse Search</b>.</p>	
2	<p>Click <b>User-Select</b> button</p> <p>Use button <b>Remove All</b> to clean current database selection</p> <p>Then, use <b>Select by Browsing</b> button to add the example database: C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Mixture Analysis\MS Examples\Components.SDBX</p> <p><b>Search</b></p>	

Action	Result																																								
<p>3 In <b>Minelt</b>, make sure that <b>Subtract View</b> (circled) is selected.</p> <p>In the <b>spectrumPane</b>, the first row is the unknown, the last row is the reference spectrum, and the middle row is the difference between the two.</p>	 <p>The screenshot shows the Minelt software interface. At the top, there is a toolbar with various icons, and a search bar labeled 'Lookup Compound:'. Below the toolbar, there are three stacked mass spectra plots. The top plot is labeled '- MSMixtu #1; MX1 - 50% Methyl isocyanide + 50% Behenyl palmitate'. The middle plot is labeled '- Difference Spectrum'. The bottom plot is labeled '- Compoen #17; Methyl isocyanide'. The x-axis for all plots is 'm/z' ranging from 0 to 550. Below the plots, there is a table with the following data:</p> <table border="1"> <thead> <tr> <th></th> <th>R.HQI</th> <th>HQI</th> <th>Tag</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>987.99</td> <td>57.44</td> <td>mpoi</td> <td>17</td> <td></td> <td>Methyl isocyanide</td> <td></td> </tr> <tr> <td>2</td> <td>978.24</td> <td>970.04</td> <td>mpoi</td> <td>21</td> <td></td> <td>Behenyl palmitate</td> <td></td> </tr> <tr> <td>3</td> <td>673.90</td> <td>292.00</td> <td>mpoi</td> <td>7</td> <td></td> <td>2-Isononenal</td> <td></td> </tr> <tr> <td>4</td> <td>665.67</td> <td>397.04</td> <td>mpoi</td> <td>1</td> <td></td> <td>(Z)-11-Tetradecenal</td> <td></td> </tr> </tbody> </table> <p>The hits are initially sorted by the <b>Reverse Search HQI (R.HQI)</b>.</p>		R.HQI	HQI	Tag	DB	ID	Name	Spectrum	1	987.99	57.44	mpoi	17		Methyl isocyanide		2	978.24	970.04	mpoi	21		Behenyl palmitate		3	673.90	292.00	mpoi	7		2-Isononenal		4	665.67	397.04	mpoi	1		(Z)-11-Tetradecenal	
	R.HQI	HQI	Tag	DB	ID	Name	Spectrum																																		
1	987.99	57.44	mpoi	17		Methyl isocyanide																																			
2	978.24	970.04	mpoi	21		Behenyl palmitate																																			
3	673.90	292.00	mpoi	7		2-Isononenal																																			
4	665.67	397.04	mpoi	1		(Z)-11-Tetradecenal																																			

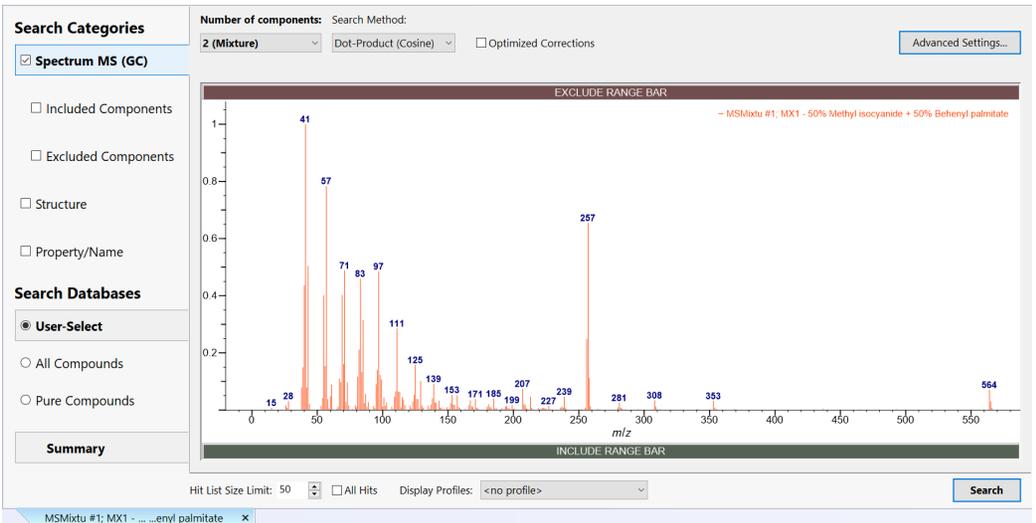
	Action	Result
4	<p>Transfer spectra in <b>spectrumPane</b> to <b>SearchIt</b> using the Transfer to: menu at the top of the application.</p> <p>Select to transfer only the <b>Difference Spectrum</b>. Click <b>OK</b>.</p> <p>When prompted by <b>SearchIt</b>, choose <b>Start a new search</b>.</p>	

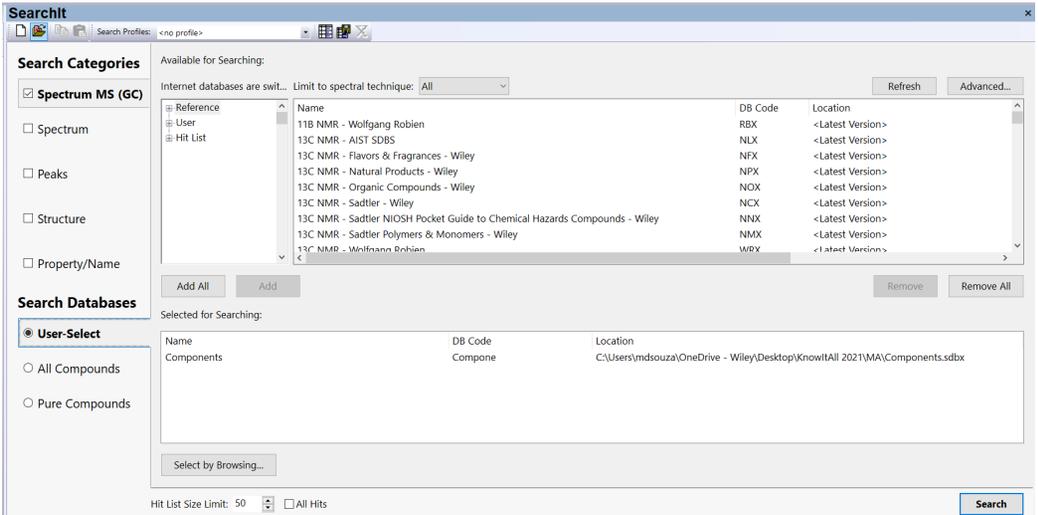
	Action	Result
5	Click <b>Search</b> .	 <p>The screenshot displays the SearchIt software interface. On the left, there are search categories and databases. The main area shows a mass spectrum plot titled 'Difference Spectrum' with the x-axis labeled 'm/z' ranging from 0 to 550. The y-axis represents relative intensity from 0 to 1.0. A prominent peak is labeled at m/z 57. Other labeled peaks include 43, 71, 83, 97, 111, 125, 139, 153, 171, 185, 199, 207, 227, 239, 257, 281, 308, 353, and 564. The interface also includes search parameters like 'Number of components: 1 (Single)', 'Search Method: Dot-Product (Cosine)', and a 'Search' button at the bottom right.</p>

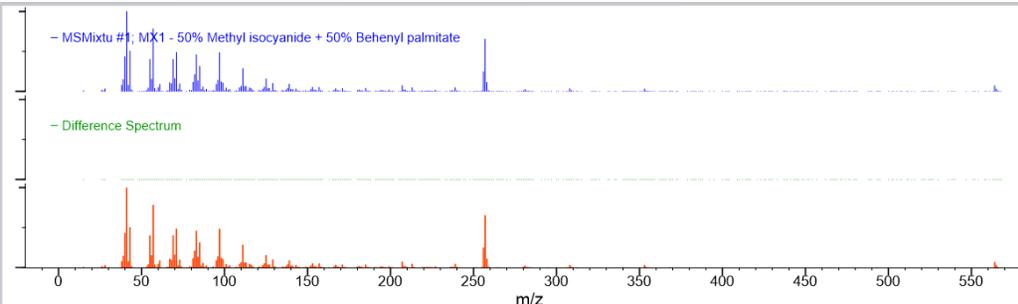
	Action	Result																																				
5		 <p>MS (GC)</p> <table border="1" data-bbox="909 695 1938 865"> <thead> <tr> <th colspan="2">Table</th> <th colspan="2">Plot</th> <th colspan="3">Related Compounds View</th> <th></th> <th></th> </tr> <tr> <th>R.HQI</th> <th>HQI</th> <th>Tag</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>&lt;auto&gt;</th> <th>(MS (GC))</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>991.32</td> <td>991.32</td> <td>mpo</td> <td>21</td> <td>Behenyl palmitate</td> <td></td> <td></td> <td></td> </tr> <tr> <td>2</td> <td>680.09</td> <td>449.83</td> <td>mpo</td> <td>38</td> <td>Octatriacontane</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <p>A second component is identified.</p> <p>Notice that the <b>Difference Spectrum</b> is empty, which means there are no more components.</p>	Table		Plot		Related Compounds View					R.HQI	HQI	Tag	DB	ID	Name	Spectrum	<auto>	(MS (GC))	1	991.32	991.32	mpo	21	Behenyl palmitate				2	680.09	449.83	mpo	38	Octatriacontane			
Table		Plot		Related Compounds View																																		
R.HQI	HQI	Tag	DB	ID	Name	Spectrum	<auto>	(MS (GC))																														
1	991.32	991.32	mpo	21	Behenyl palmitate																																	
2	680.09	449.83	mpo	38	Octatriacontane																																	

## Mixture Analysis

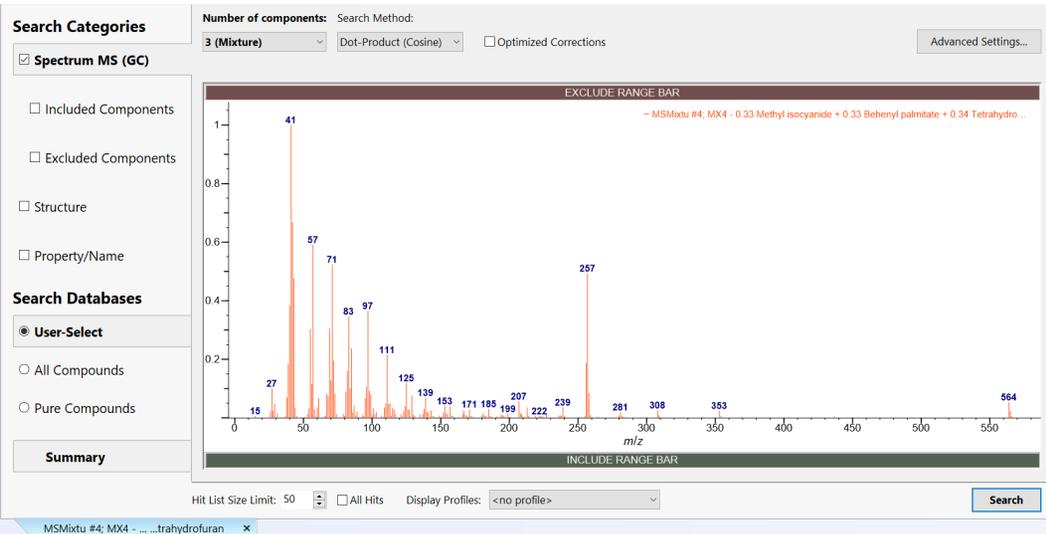
A mixture wherein two-component MS spectra have limited overlap, and one of them has a large MS range

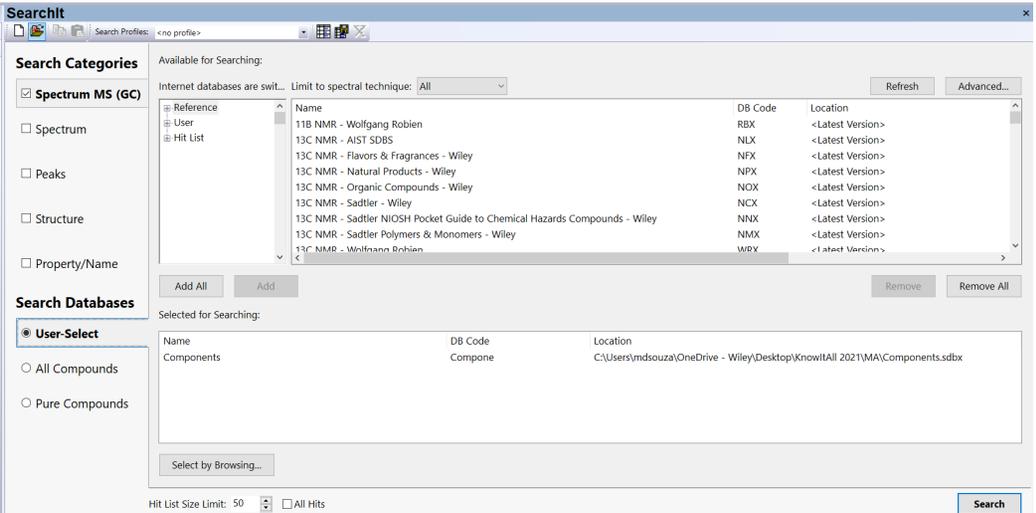
	Action	Result
1	<p>Go back to <b>SearchIt</b>.</p> <p>Start a new search by clicking .</p> <p>Select <b>MS Mixture of Two 1</b>.</p> <p>Ensure <b>Reverse Search</b> or <b>Adaptive Search</b> is unchecked.</p> <p>Use the drop-down menu to set the <b>Number of components</b> to <b>2</b>.</p>	 <p>The screenshot shows the SearchIt interface with the following details:</p> <ul style="list-style-type: none"> <li><b>Search Categories:</b> Spectrum MS (GC) is selected. Other categories like Included Components, Excluded Components, Structure, and Property/Name are unchecked.</li> <li><b>Search Databases:</b> User-Select is selected. All Compounds and Pure Compounds are unselected.</li> <li><b>Search Parameters:</b> Number of components is set to 2 (Mixture). Search Method is Dot-Product (Cosine). Optimized Corrections is unchecked.</li> <li><b>Mass Spectrum:</b> The plot shows relative intensity versus m/z. The x-axis ranges from 0 to 550 m/z. The y-axis ranges from 0 to 1.0. Major peaks are labeled at m/z 41, 57, 71, 83, 97, 111, 125, 139, 153, 171, 185, 199, 207, 227, 239, 257, 281, 308, 353, and 564. A legend indicates the mixture is 50% Methyl isocyanide + 50% Behenyl palmitate.</li> <li><b>Controls:</b> Hit List Size Limit is 50. All Hits is unchecked. Display Profiles is set to &lt;no profile&gt;. A Search button is present at the bottom right.</li> </ul>

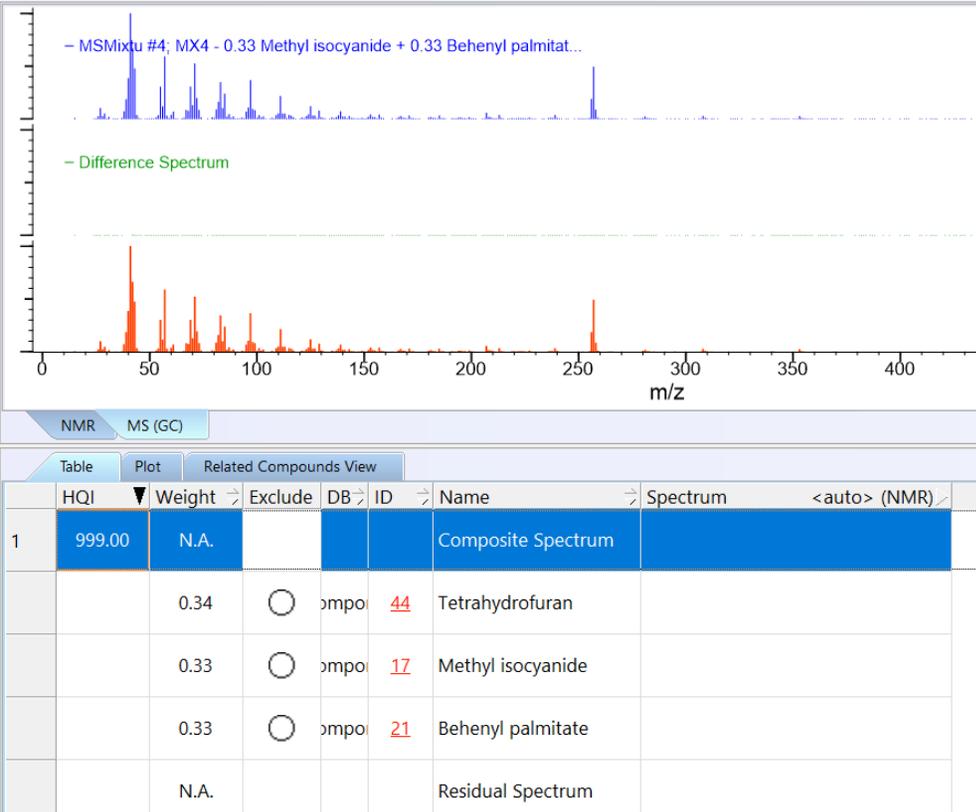
	Action	Result																														
2	<p>Click on <b>User-Select</b>.</p> <p>Make sure <b>Components</b> database is selected to use.</p> <p>Click <b>Search</b>.</p>	 <p>The screenshot shows the SearchIt application window. On the left, under 'Search Categories', 'Spectrum MS (GC)' is selected. Under 'Search Databases', 'User-Select' is selected, and 'All Compounds' is chosen. The main area displays a table of available databases for searching.</p> <table border="1" data-bbox="848 418 1709 586"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>11B NMR - Wolfgang Robien</td> <td>RBX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - AIST SDBS</td> <td>NLX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Flavors &amp; Fragrances - Wiley</td> <td>NFX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Natural Products - Wiley</td> <td>NPX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Organic Compounds - Wiley</td> <td>NOX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Sadtler - Wiley</td> <td>NCK</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td> <td>NMX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Sadtler Polymers &amp; Monomers - Wiley</td> <td>NMX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td>13C NMR - Wolfmann Bohlen</td> <td>WBY</td> <td>&lt;Latest Versions&gt;</td> </tr> </tbody> </table> <p>Below this table, the 'Selected for Searching' section shows the 'Components' database selected, with its DB Code 'Compone' and Location 'C:\Users\mdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx'.</p>	Name	DB Code	Location	11B NMR - Wolfgang Robien	RBX	<Latest Versions>	13C NMR - AIST SDBS	NLX	<Latest Versions>	13C NMR - Flavors & Fragrances - Wiley	NFX	<Latest Versions>	13C NMR - Natural Products - Wiley	NPX	<Latest Versions>	13C NMR - Organic Compounds - Wiley	NOX	<Latest Versions>	13C NMR - Sadtler - Wiley	NCK	<Latest Versions>	13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NMX	<Latest Versions>	13C NMR - Sadtler Polymers & Monomers - Wiley	NMX	<Latest Versions>	13C NMR - Wolfmann Bohlen	WBY	<Latest Versions>
Name	DB Code	Location																														
11B NMR - Wolfgang Robien	RBX	<Latest Versions>																														
13C NMR - AIST SDBS	NLX	<Latest Versions>																														
13C NMR - Flavors & Fragrances - Wiley	NFX	<Latest Versions>																														
13C NMR - Natural Products - Wiley	NPX	<Latest Versions>																														
13C NMR - Organic Compounds - Wiley	NOX	<Latest Versions>																														
13C NMR - Sadtler - Wiley	NCK	<Latest Versions>																														
13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NMX	<Latest Versions>																														
13C NMR - Sadtler Polymers & Monomers - Wiley	NMX	<Latest Versions>																														
13C NMR - Wolfmann Bohlen	WBY	<Latest Versions>																														

Action	Result																																												
	 <p data-bbox="693 633 1711 665">NMR MS (GC)</p> <table border="1" data-bbox="693 673 1711 966"> <thead> <tr> <th>Table</th> <th>Plot</th> <th colspan="2">Related Compounds View</th> </tr> <tr> <th>HQI</th> <th>Weight</th> <th>Exclude</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>&lt;auto&gt; (NMR)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>999.00</td> <td>N.A.</td> <td></td> <td></td> <td>Composite Spectrum</td> <td></td> <td></td> </tr> <tr> <td></td> <td>0.50</td> <td><input type="radio"/></td> <td>mpo</td> <td>17</td> <td>Methyl isocyanide</td> <td></td> <td></td> </tr> <tr> <td></td> <td>0.50</td> <td><input type="radio"/></td> <td>mpo</td> <td>21</td> <td>Behenyl palmitate</td> <td></td> <td></td> </tr> <tr> <td></td> <td>N.A.</td> <td></td> <td></td> <td></td> <td>Residual Spectrum</td> <td></td> <td></td> </tr> </tbody> </table> <p data-bbox="682 1063 1732 1153">KnowItAll presents a 2-component search result. The top spectrum is unknown; the bottom spectrum is the composite spectrum of 2 components. The middle spectrum is the difference between the two. In this case, it is next to nothing, indicating that there are no more residual peaks.</p> <p data-bbox="682 1185 1732 1242"><b>Note:</b> This is an example wherein the two-component MS spectra have limited overlap, but one of them has large MS range.</p>	Table	Plot	Related Compounds View		HQI	Weight	Exclude	DB	ID	Name	Spectrum	<auto> (NMR)	1	999.00	N.A.			Composite Spectrum				0.50	<input type="radio"/>	mpo	17	Methyl isocyanide				0.50	<input type="radio"/>	mpo	21	Behenyl palmitate				N.A.				Residual Spectrum		
Table	Plot	Related Compounds View																																											
HQI	Weight	Exclude	DB	ID	Name	Spectrum	<auto> (NMR)																																						
1	999.00	N.A.			Composite Spectrum																																								
	0.50	<input type="radio"/>	mpo	17	Methyl isocyanide																																								
	0.50	<input type="radio"/>	mpo	21	Behenyl palmitate																																								
	N.A.				Residual Spectrum																																								

### A more complex example

	Action	Result
1	<p>Go back to <b>SearchIt</b>.</p> <p>Start a new search by clicking .</p> <p>Select the file <b>MS Mixture of Three</b>.</p> <p>Use the drop-down menu to set the <b>Number of components to 3 (Mixture)</b>.</p>	 <p><b>Search Categories</b></p> <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Spectrum MS (GC)</li> <li><input type="checkbox"/> Included Components</li> <li><input type="checkbox"/> Excluded Components</li> <li><input type="checkbox"/> Structure</li> <li><input type="checkbox"/> Property/Name</li> </ul> <p><b>Search Databases</b></p> <ul style="list-style-type: none"> <li><input checked="" type="radio"/> User-Select</li> <li><input type="radio"/> All Compounds</li> <li><input type="radio"/> Pure Compounds</li> </ul> <p><b>Summary</b></p> <p>Number of components: 3 (Mixture) Search Method: Dot-Product (Cosine) <input type="checkbox"/> Optimized Corrections <a href="#">Advanced Settings...</a></p> <p>Hit List Size Limit: 50 <input type="checkbox"/> All Hits Display Profiles: &lt;no profile&gt; <a href="#">Search</a></p> <p>MSMixture #4; MX4 - ...trahydrofuran</p>

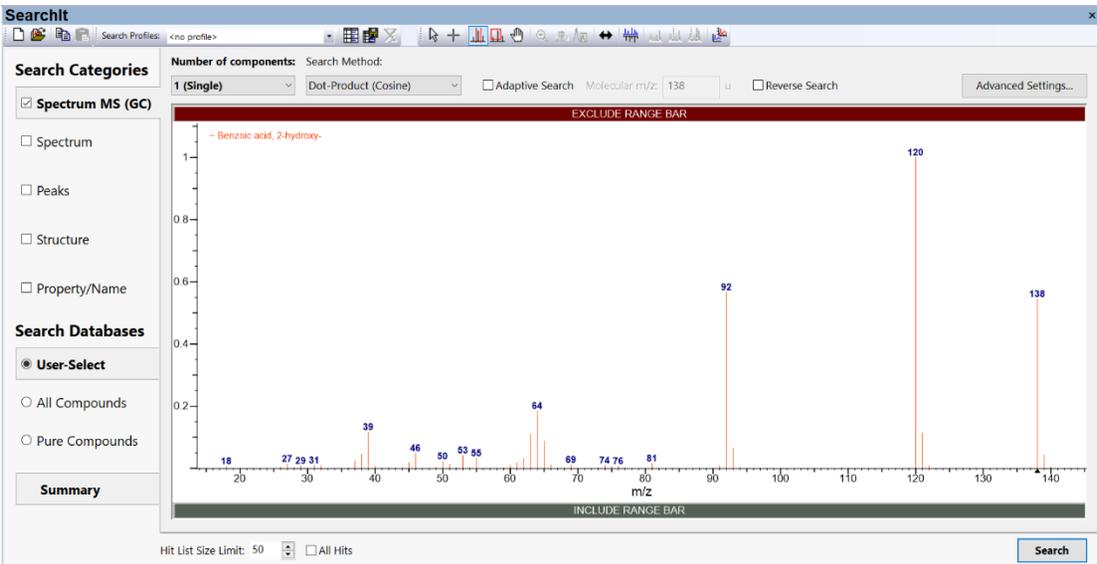
	Action	Result																																														
2	<p>Click on <b>User-Select</b>.</p> <p>Make sure the <b>Components</b> database is selected for use.</p> <p>Click <b>Search</b>.</p>	 <p>The screenshot shows the SearchIt application window. On the left, under 'Search Categories', 'Spectrum MS (GC)' is checked. Under 'Search Databases', 'User-Select' is selected. The 'Selected for Searching' table shows the 'Components' database selected.</p> <table border="1" data-bbox="842 418 1709 586"> <thead> <tr> <th>Reference</th> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td></td> <td>11B NMR - Wolfgang Robien</td> <td>RBX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - AIST SDBS</td> <td>NLX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Flavors &amp; Fragrances - Wiley</td> <td>NFX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Natural Products - Wiley</td> <td>NPX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Organic Compounds - Wiley</td> <td>NOX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Sadtler - Wiley</td> <td>NXX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td> <td>NMX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Sadtler Polymers &amp; Monomers - Wiley</td> <td>NMX</td> <td>&lt;Latest Versions&gt;</td> </tr> <tr> <td></td> <td>13C NMR - Wolfmann Bohlen</td> <td>WBY</td> <td>&lt;Latest Versions&gt;</td> </tr> </tbody> </table> <table border="1" data-bbox="842 651 1709 760"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>Components</td> <td>Compone</td> <td>C:\Users\mdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx</td> </tr> </tbody> </table>	Reference	Name	DB Code	Location		11B NMR - Wolfgang Robien	RBX	<Latest Versions>		13C NMR - AIST SDBS	NLX	<Latest Versions>		13C NMR - Flavors & Fragrances - Wiley	NFX	<Latest Versions>		13C NMR - Natural Products - Wiley	NPX	<Latest Versions>		13C NMR - Organic Compounds - Wiley	NOX	<Latest Versions>		13C NMR - Sadtler - Wiley	NXX	<Latest Versions>		13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NMX	<Latest Versions>		13C NMR - Sadtler Polymers & Monomers - Wiley	NMX	<Latest Versions>		13C NMR - Wolfmann Bohlen	WBY	<Latest Versions>	Name	DB Code	Location	Components	Compone	C:\Users\mdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx
Reference	Name	DB Code	Location																																													
	11B NMR - Wolfgang Robien	RBX	<Latest Versions>																																													
	13C NMR - AIST SDBS	NLX	<Latest Versions>																																													
	13C NMR - Flavors & Fragrances - Wiley	NFX	<Latest Versions>																																													
	13C NMR - Natural Products - Wiley	NPX	<Latest Versions>																																													
	13C NMR - Organic Compounds - Wiley	NOX	<Latest Versions>																																													
	13C NMR - Sadtler - Wiley	NXX	<Latest Versions>																																													
	13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NMX	<Latest Versions>																																													
	13C NMR - Sadtler Polymers & Monomers - Wiley	NMX	<Latest Versions>																																													
	13C NMR - Wolfmann Bohlen	WBY	<Latest Versions>																																													
Name	DB Code	Location																																														
Components	Compone	C:\Users\mdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx																																														

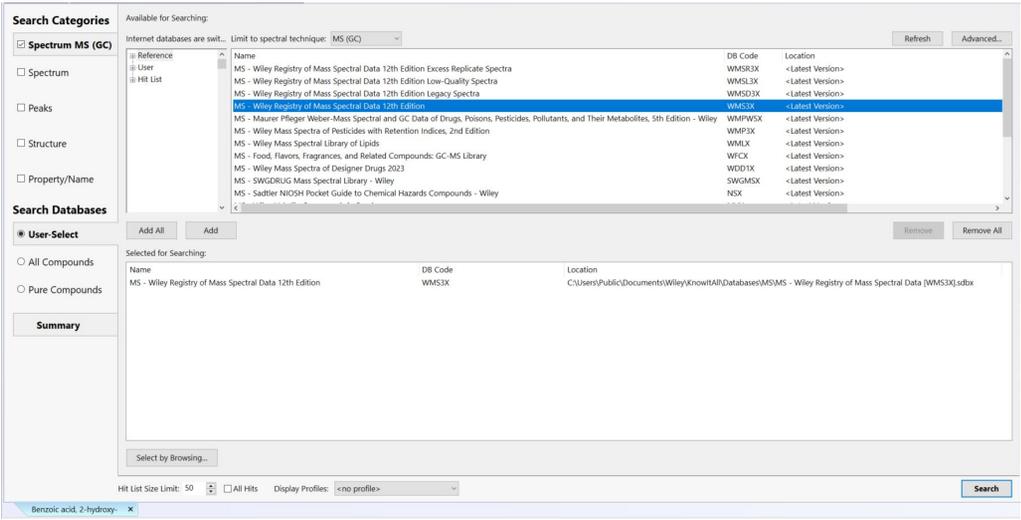
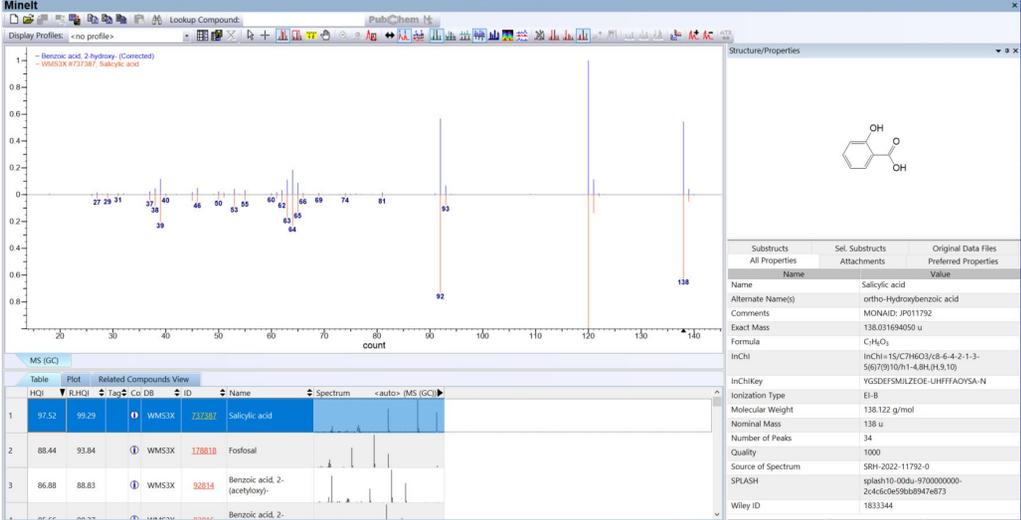
Action	Result																																										
	 <table border="1" data-bbox="693 803 1669 1136"> <thead> <tr> <th>HQI</th> <th>Weight</th> <th>Exclude</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>999.00</td> <td>N.A.</td> <td></td> <td></td> <td>Composite Spectrum</td> <td></td> </tr> <tr> <td></td> <td>0.34</td> <td><input type="radio"/></td> <td>compo</td> <td>44</td> <td>Tetrahydrofuran</td> <td></td> </tr> <tr> <td></td> <td>0.33</td> <td><input type="radio"/></td> <td>compo</td> <td>17</td> <td>Methyl isocyanide</td> <td></td> </tr> <tr> <td></td> <td>0.33</td> <td><input type="radio"/></td> <td>compo</td> <td>21</td> <td>Behenyl palmitate</td> <td></td> </tr> <tr> <td></td> <td>N.A.</td> <td></td> <td></td> <td></td> <td>Residual Spectrum</td> <td></td> </tr> </tbody> </table> <p>KnowItAll presents a 3-component search result. The top spectrum is unknown; the bottom spectrum is the composite spectrum of 3 components. The middle spectrum is the difference between the two. In this case, it is next to nothing, indicating that there are no more residual peaks.</p> <p>This process accomplishes many steps in one. It also avoids negative peaks from spectral subtraction.</p>	HQI	Weight	Exclude	DB	ID	Name	Spectrum	1	999.00	N.A.			Composite Spectrum			0.34	<input type="radio"/>	compo	44	Tetrahydrofuran			0.33	<input type="radio"/>	compo	17	Methyl isocyanide			0.33	<input type="radio"/>	compo	21	Behenyl palmitate			N.A.				Residual Spectrum	
HQI	Weight	Exclude	DB	ID	Name	Spectrum																																					
1	999.00	N.A.			Composite Spectrum																																						
	0.34	<input type="radio"/>	compo	44	Tetrahydrofuran																																						
	0.33	<input type="radio"/>	compo	17	Methyl isocyanide																																						
	0.33	<input type="radio"/>	compo	21	Behenyl palmitate																																						
	N.A.				Residual Spectrum																																						

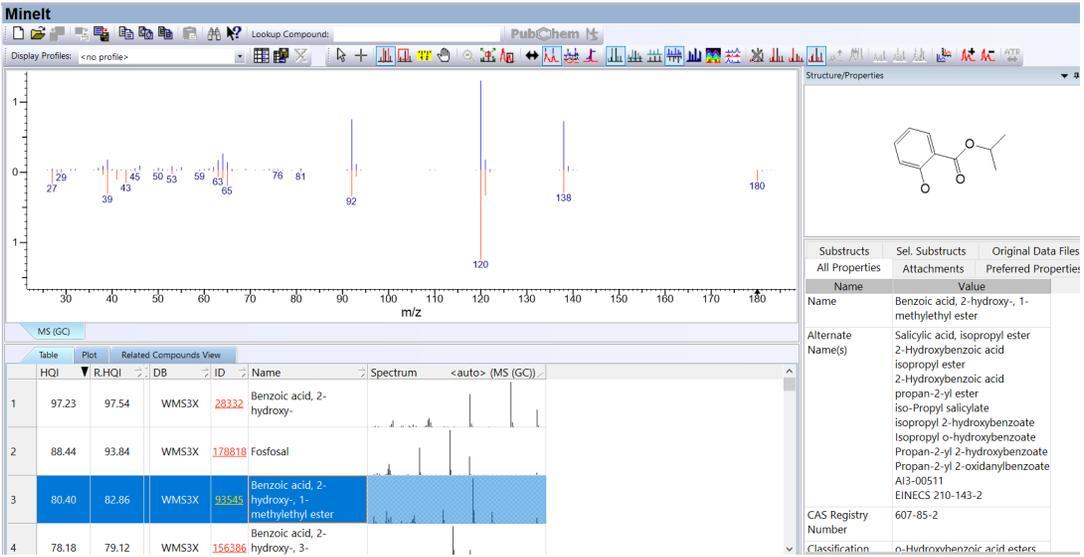
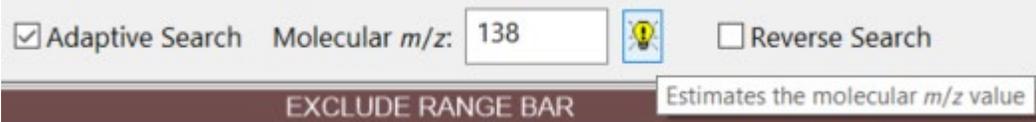
## Adaptive Search

This search finds similar compounds where a fragment group can be present or missing compared to the unknown. The presence/absence of a fragment causes some peak positions in reference MS differing to that of unknown by a delta mass ( $\Delta m$ ). KnowItAll shifts some peaks by the  $\Delta m$  to achieve a better matching score. Because of the better matching score, similar compounds come atop of the hit list. To clearly mark the shifts done by Adaptive Search, dotted lines are used to show reference spectrum before and after shifting in the pop-up window when you click on the (i) button in a hit.

### Example wherein exact mass is in a spectrum

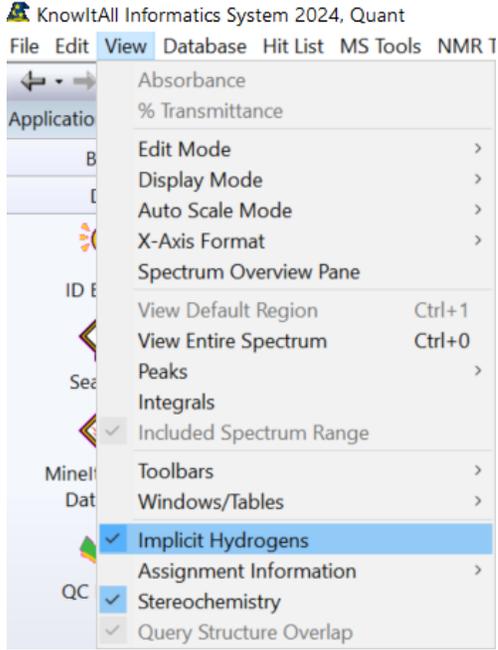
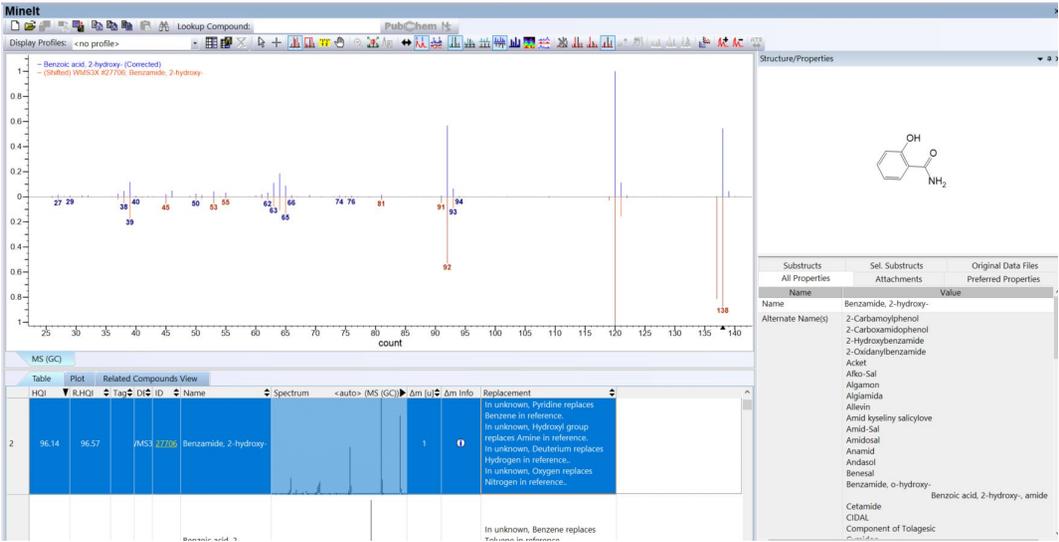
	Action	Result
1	<p>Start a new search by clicking .</p> <p>Click <b>Spectrum</b> to navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS folder</p> <p>Select the file <b>2-Hydroxybenzoic acid</b>.</p> <p>Use the drop-down menu to set the <b>Number of components to 1 (Single)</b>.</p> <p>Ensure <b>Adaptive Search</b> or <b>Reverse Search</b> is unchecked.</p>	 <p>This spectrum file contains a molecular ion mass of 138. KnowItAll will use this value for the Adaptive Search.</p>

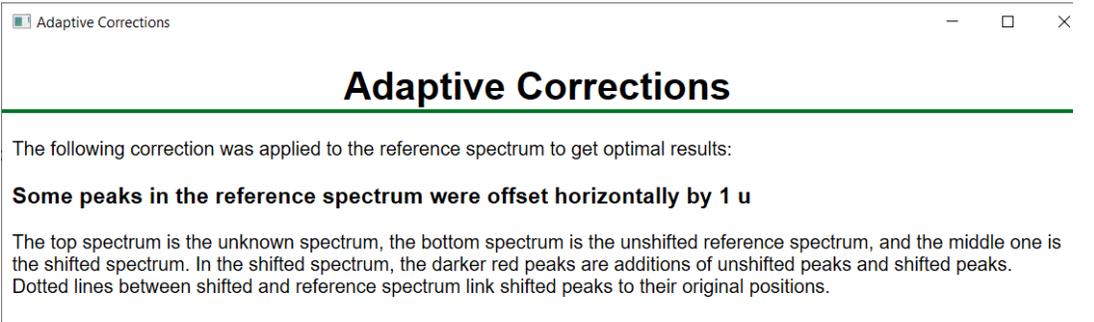
Action	Result																																																																																		
<p>2 Click <b>User-Select</b> button</p> <p>Use <b>Remove All</b> to clean selected databases.</p> <p>Use <b>Add</b> to add MS (GC) databases by codes: <b>WMS3X</b>.</p> <p>Click <b>Search</b>.</p>	 <p><b>Search Categories</b></p> <p>Available for Searching: Limit to spectral technique: MS (GC)</p> <p>Internet databases are swi... Refresh Advanced...</p> <table border="1"> <thead> <tr> <th>Reference</th> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>MS - Wiley Registry of Mass Spectral Data 12th Edition Excess Replicate Spectra</td> <td></td> <td>WMSR3X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Wiley Registry of Mass Spectral Data 12th Edition Low-Quality Spectra</td> <td></td> <td>WMSL3X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Wiley Registry of Mass Spectral Data 12th Edition Legacy Spectra</td> <td></td> <td>WMSL3X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Wiley Registry of Mass Spectral Data 12th Edition</td> <td></td> <td>WMS3X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Maurer Pfeleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites, 5th Edition - Wiley</td> <td></td> <td>WMPW5X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Wiley Mass Spectra of Pesticides with Retention Indices, 2nd Edition</td> <td></td> <td>WMP3X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Wiley Mass Spectral Library of Lipids</td> <td></td> <td>WMLX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Food, Flavors, Fragrances, and Related Compounds: GC-MS Library</td> <td></td> <td>WFCX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Wiley Mass Spectra of Designer Drugs 2023</td> <td></td> <td>WDD1X</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - SWGDRLUG Mass Spectral Library - Wiley</td> <td></td> <td>SWGMSX</td> <td>&lt;Latest Version&gt;</td> </tr> <tr> <td>MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td> <td></td> <td>NSX</td> <td>&lt;Latest Version&gt;</td> </tr> </tbody> </table> <p><b>Search Databases</b></p> <p>Selected for Searching:</p> <table border="1"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>MS - Wiley Registry of Mass Spectral Data 12th Edition</td> <td>WMS3X</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Registry of Mass Spectral Data (WMS3X).sdbx</td> </tr> </tbody> </table> <p>Buttons: Add All, Add, Remove, Remove All</p> <p>Summary</p> <p>Select by Browsing...</p> <p>Hit List Size Limit: 50 All Hits Display Profiles: &lt;no profile&gt; Search</p> <p>Benzoic acid, 2-hydroxy-</p>	Reference	Name	DB Code	Location	MS - Wiley Registry of Mass Spectral Data 12th Edition Excess Replicate Spectra		WMSR3X	<Latest Version>	MS - Wiley Registry of Mass Spectral Data 12th Edition Low-Quality Spectra		WMSL3X	<Latest Version>	MS - Wiley Registry of Mass Spectral Data 12th Edition Legacy Spectra		WMSL3X	<Latest Version>	MS - Wiley Registry of Mass Spectral Data 12th Edition		WMS3X	<Latest Version>	MS - Maurer Pfeleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites, 5th Edition - Wiley		WMPW5X	<Latest Version>	MS - Wiley Mass Spectra of Pesticides with Retention Indices, 2nd Edition		WMP3X	<Latest Version>	MS - Wiley Mass Spectral Library of Lipids		WMLX	<Latest Version>	MS - Food, Flavors, Fragrances, and Related Compounds: GC-MS Library		WFCX	<Latest Version>	MS - Wiley Mass Spectra of Designer Drugs 2023		WDD1X	<Latest Version>	MS - SWGDRLUG Mass Spectral Library - Wiley		SWGMSX	<Latest Version>	MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley		NSX	<Latest Version>	Name	DB Code	Location	MS - Wiley Registry of Mass Spectral Data 12th Edition	WMS3X	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Registry of Mass Spectral Data (WMS3X).sdbx																												
Reference	Name	DB Code	Location																																																																																
MS - Wiley Registry of Mass Spectral Data 12th Edition Excess Replicate Spectra		WMSR3X	<Latest Version>																																																																																
MS - Wiley Registry of Mass Spectral Data 12th Edition Low-Quality Spectra		WMSL3X	<Latest Version>																																																																																
MS - Wiley Registry of Mass Spectral Data 12th Edition Legacy Spectra		WMSL3X	<Latest Version>																																																																																
MS - Wiley Registry of Mass Spectral Data 12th Edition		WMS3X	<Latest Version>																																																																																
MS - Maurer Pfeleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, and Their Metabolites, 5th Edition - Wiley		WMPW5X	<Latest Version>																																																																																
MS - Wiley Mass Spectra of Pesticides with Retention Indices, 2nd Edition		WMP3X	<Latest Version>																																																																																
MS - Wiley Mass Spectral Library of Lipids		WMLX	<Latest Version>																																																																																
MS - Food, Flavors, Fragrances, and Related Compounds: GC-MS Library		WFCX	<Latest Version>																																																																																
MS - Wiley Mass Spectra of Designer Drugs 2023		WDD1X	<Latest Version>																																																																																
MS - SWGDRLUG Mass Spectral Library - Wiley		SWGMSX	<Latest Version>																																																																																
MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley		NSX	<Latest Version>																																																																																
Name	DB Code	Location																																																																																	
MS - Wiley Registry of Mass Spectral Data 12th Edition	WMS3X	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Registry of Mass Spectral Data (WMS3X).sdbx																																																																																	
<p>3 Click the <b>Butterfly view</b> icon .</p>	 <p><b>Minet</b></p> <p>Lookup Compound: PubChem</p> <p>Display Profiles: &lt;no profile&gt;</p> <p>Structure/Properties</p> <p>Subtracts All Properties Sel. Subtracts Attachments Original Data Files Preferred Properties Value</p> <table border="1"> <thead> <tr> <th>Name</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td>Salicylic acid</td> </tr> <tr> <td>Alternate Name(s)</td> <td>ortho-Hydroxybenzoic acid</td> </tr> <tr> <td>Comments</td> <td>MOLNAID: J011792</td> </tr> <tr> <td>Exact Mass</td> <td>138.03164050 u</td> </tr> <tr> <td>Formula</td> <td>C<sub>7</sub>H<sub>6</sub>O<sub>3</sub></td> </tr> <tr> <td>InChI</td> <td>InChI=1S/C7H6O3/c8-6-4-2-1-3-5(6)/7910/h1-4,8H,(H3,10)</td> </tr> <tr> <td>InChIKey</td> <td>YGSDFSMJZEEU-LHFFFAOYSA-N</td> </tr> <tr> <td>Ionization Type</td> <td>E-I</td> </tr> <tr> <td>Molecular Weight</td> <td>138.122 g/mol</td> </tr> <tr> <td>Nominal Mass</td> <td>138 u</td> </tr> <tr> <td>Number of Peaks</td> <td>34</td> </tr> <tr> <td>Quality</td> <td>1000</td> </tr> <tr> <td>Source of Spectrum</td> <td>SRH-2022-11792-0</td> </tr> <tr> <td>SPLASH</td> <td>splash10-00du-970000000-2c4c6e596b8947e873</td> </tr> <tr> <td>Wiley ID</td> <td>183344</td> </tr> </tbody> </table> <p>MS (GC)</p> <table border="1"> <thead> <tr> <th>HC#</th> <th>R#H#</th> <th>Top</th> <th>CU</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>&lt;auto&gt;</th> <th>(MS (GC))</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>97.52</td> <td>99.29</td> <td></td> <td>WMS3X</td> <td>737381</td> <td>Salicylic acid</td> <td></td> <td></td> <td></td> </tr> <tr> <td>2</td> <td>88.44</td> <td>93.84</td> <td></td> <td>WMS3X</td> <td>178818</td> <td>Fosfosal</td> <td></td> <td></td> <td></td> </tr> <tr> <td>3</td> <td>86.88</td> <td>88.83</td> <td></td> <td>WMS3X</td> <td>92814</td> <td>Benzoic acid, 2-(acetyloxy)-</td> <td></td> <td></td> <td></td> </tr> <tr> <td>4</td> <td>86.66</td> <td>88.73</td> <td></td> <td>WMS3X</td> <td>92814</td> <td>Benzoic acid, 2-</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>	Name	Value	Name	Salicylic acid	Alternate Name(s)	ortho-Hydroxybenzoic acid	Comments	MOLNAID: J011792	Exact Mass	138.03164050 u	Formula	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	InChI	InChI=1S/C7H6O3/c8-6-4-2-1-3-5(6)/7910/h1-4,8H,(H3,10)	InChIKey	YGSDFSMJZEEU-LHFFFAOYSA-N	Ionization Type	E-I	Molecular Weight	138.122 g/mol	Nominal Mass	138 u	Number of Peaks	34	Quality	1000	Source of Spectrum	SRH-2022-11792-0	SPLASH	splash10-00du-970000000-2c4c6e596b8947e873	Wiley ID	183344	HC#	R#H#	Top	CU	DB	ID	Name	Spectrum	<auto>	(MS (GC))	1	97.52	99.29		WMS3X	737381	Salicylic acid				2	88.44	93.84		WMS3X	178818	Fosfosal				3	86.88	88.83		WMS3X	92814	Benzoic acid, 2-(acetyloxy)-				4	86.66	88.73		WMS3X	92814	Benzoic acid, 2-			
Name	Value																																																																																		
Name	Salicylic acid																																																																																		
Alternate Name(s)	ortho-Hydroxybenzoic acid																																																																																		
Comments	MOLNAID: J011792																																																																																		
Exact Mass	138.03164050 u																																																																																		
Formula	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>																																																																																		
InChI	InChI=1S/C7H6O3/c8-6-4-2-1-3-5(6)/7910/h1-4,8H,(H3,10)																																																																																		
InChIKey	YGSDFSMJZEEU-LHFFFAOYSA-N																																																																																		
Ionization Type	E-I																																																																																		
Molecular Weight	138.122 g/mol																																																																																		
Nominal Mass	138 u																																																																																		
Number of Peaks	34																																																																																		
Quality	1000																																																																																		
Source of Spectrum	SRH-2022-11792-0																																																																																		
SPLASH	splash10-00du-970000000-2c4c6e596b8947e873																																																																																		
Wiley ID	183344																																																																																		
HC#	R#H#	Top	CU	DB	ID	Name	Spectrum	<auto>	(MS (GC))																																																																										
1	97.52	99.29		WMS3X	737381	Salicylic acid																																																																													
2	88.44	93.84		WMS3X	178818	Fosfosal																																																																													
3	86.88	88.83		WMS3X	92814	Benzoic acid, 2-(acetyloxy)-																																																																													
4	86.66	88.73		WMS3X	92814	Benzoic acid, 2-																																																																													

Action	Result																																								
<p>4 Highlight the 2<sup>nd</sup> then the 3<sup>rd</sup> hit.</p>	 <p>The screenshot shows the Minelt software interface. At the top, there is a search bar and a toolbar. Below that is a mass spectrum plot with the x-axis labeled 'm/z' ranging from 30 to 180 and the y-axis representing relative intensity. The base peak is at m/z 120. Below the plot is a table of search results:</p> <table border="1"> <thead> <tr> <th>HQI</th> <th>R.HQI</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>97.23</td> <td>97.54</td> <td>WMS3X</td> <td>28332 Benzoic acid, 2-hydroxy-</td> <td></td> </tr> <tr> <td>2</td> <td>88.44</td> <td>93.84</td> <td>WMS3X</td> <td>178818 Fosfosal</td> <td></td> </tr> <tr> <td>3</td> <td>80.40</td> <td>82.86</td> <td>WMS3X</td> <td>93545 Benzoic acid, 2-hydroxy-, 1-methylethyl ester</td> <td></td> </tr> <tr> <td>4</td> <td>78.18</td> <td>79.12</td> <td>WMS3X</td> <td>156386 Benzoic acid, 2-hydroxy-, 3-</td> <td></td> </tr> </tbody> </table> <p>Below the table is a 'Structure/Properties' panel showing the chemical structure of the 3rd hit and a list of properties:</p> <table border="1"> <thead> <tr> <th>Name</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td>Benzoic acid, 2-hydroxy-, 1-methylethyl ester</td> </tr> <tr> <td>Alternate Name(s)</td> <td>Salicylic acid, isopropyl ester 2-Hydroxybenzoic acid isopropyl ester 2-Hydroxybenzoic acid propan-2-yl ester iso-Propyl salicylate isopropyl 2-hydroxybenzoate isopropyl o-hydroxybenzoate Propan-2-yl 2-hydroxybenzoate Propan-2-yl 2-oxidanybenzoate A13-00511 EINECS 210-143-2</td> </tr> <tr> <td>CAS Registry Number</td> <td>607-85-2</td> </tr> <tr> <td>Classification</td> <td>o-Hydroxybenzoic acid esters</td> </tr> </tbody> </table>	HQI	R.HQI	DB	ID	Name	Spectrum	1	97.23	97.54	WMS3X	28332 Benzoic acid, 2-hydroxy-		2	88.44	93.84	WMS3X	178818 Fosfosal		3	80.40	82.86	WMS3X	93545 Benzoic acid, 2-hydroxy-, 1-methylethyl ester		4	78.18	79.12	WMS3X	156386 Benzoic acid, 2-hydroxy-, 3-		Name	Value	Name	Benzoic acid, 2-hydroxy-, 1-methylethyl ester	Alternate Name(s)	Salicylic acid, isopropyl ester 2-Hydroxybenzoic acid isopropyl ester 2-Hydroxybenzoic acid propan-2-yl ester iso-Propyl salicylate isopropyl 2-hydroxybenzoate isopropyl o-hydroxybenzoate Propan-2-yl 2-hydroxybenzoate Propan-2-yl 2-oxidanybenzoate A13-00511 EINECS 210-143-2	CAS Registry Number	607-85-2	Classification	o-Hydroxybenzoic acid esters
HQI	R.HQI	DB	ID	Name	Spectrum																																				
1	97.23	97.54	WMS3X	28332 Benzoic acid, 2-hydroxy-																																					
2	88.44	93.84	WMS3X	178818 Fosfosal																																					
3	80.40	82.86	WMS3X	93545 Benzoic acid, 2-hydroxy-, 1-methylethyl ester																																					
4	78.18	79.12	WMS3X	156386 Benzoic acid, 2-hydroxy-, 3-																																					
Name	Value																																								
Name	Benzoic acid, 2-hydroxy-, 1-methylethyl ester																																								
Alternate Name(s)	Salicylic acid, isopropyl ester 2-Hydroxybenzoic acid isopropyl ester 2-Hydroxybenzoic acid propan-2-yl ester iso-Propyl salicylate isopropyl 2-hydroxybenzoate isopropyl o-hydroxybenzoate Propan-2-yl 2-hydroxybenzoate Propan-2-yl 2-oxidanybenzoate A13-00511 EINECS 210-143-2																																								
CAS Registry Number	607-85-2																																								
Classification	o-Hydroxybenzoic acid esters																																								
<p>5 Go back to <b>SearchIt</b>.</p> <p>Click <b>Spectrum MS(GC)</b></p> <p>Check <b>Adaptive Search</b>.</p> <p>Click <b>Search</b>.</p>	 <p>The screenshot shows the search interface with the following elements:</p> <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Adaptive Search</li> <li>Molecular <i>m/z</i>: <input type="text" value="138"/></li> <li></li> <li><input type="checkbox"/> Reverse Search</li> <li>EXCLUDE RANGE BAR</li> <li>Estimates the molecular <i>m/z</i> value</li> </ul>																																								

Assume that we do not have the first hit in our database. The HQI values for the 2<sup>nd</sup> and 3<sup>rd</sup> hits are not totally convincing that they are good matches.

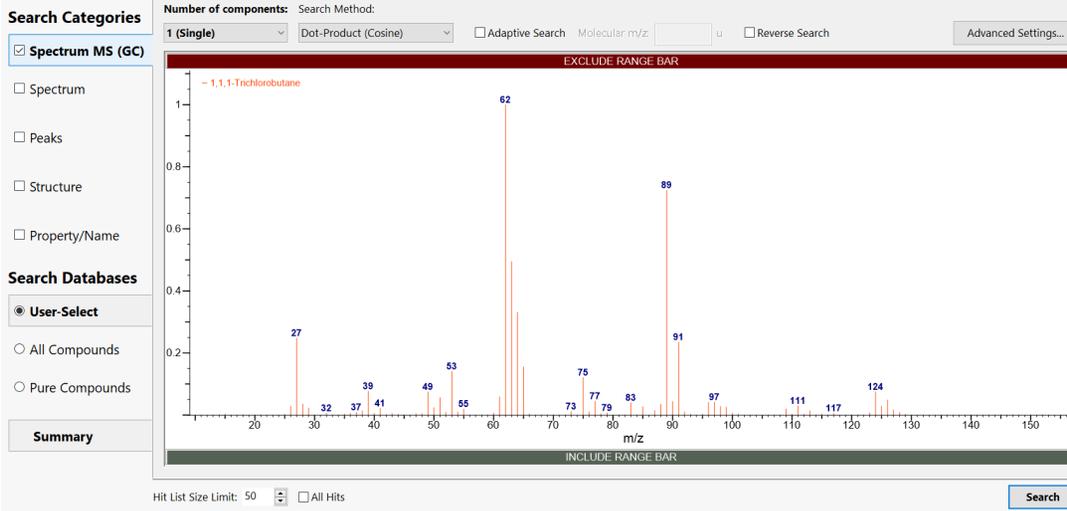
- If a spectrum file contains the molecular ion mass, it will be displayed in the "Molecular *m/z*:" box, and a solid triangle marks this position in the spectrumPane.
- You can type in an appropriate value as well. This value is used to assist Adaptive Search.
- Or, you can ask KnowItAll to estimate a molecular mass for you by clicking the bulb icon.
- However, if molecular ion mass is unknown, this box would be empty. KnowItAll Adaptive Search will estimate this value from the input unknown MS spectrum.

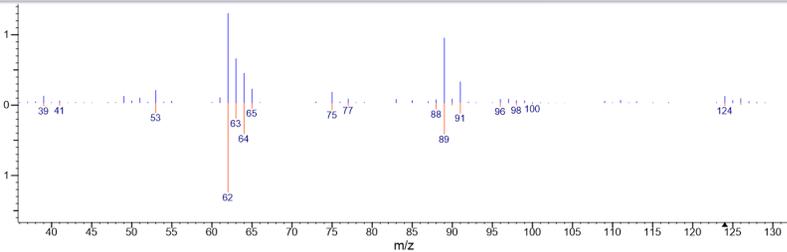
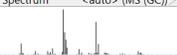
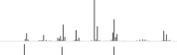
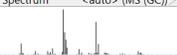
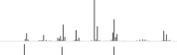
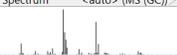
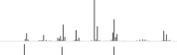
Action	Result
<p>6 Ignore the 1<sup>st</sup> hit and move to the 2<sup>nd</sup> hit.</p> <p>Make sure KnowItAll displays implicit Hydrogens, one need to go to <b>View</b> menu and check <b>Implicit Hydrogens</b></p> 	 <p>The result looks good. <math>\Delta m</math> of 1 suggests a few possibilities wherein the unknown's molecular ion mass (<math>m/z</math>) is 1 unit more than that of the reference. One of them is "In unknown, Oxygen replaces Nitrogen in reference."</p> <p>Columns of interest:</p> <ul style="list-style-type: none"> <li>• <b><math>\Delta m</math></b>: the molecular ion mass of unknown minus that of the reference</li> <li>• <b><math>\Delta m</math> Info</b>: details on reference peak shifts to make the match</li> <li>• <b>Replacement</b>: suggestions of <i>possible</i> group exchange which would cause the difference between unknown and reference molecular ion masses</li> </ul>

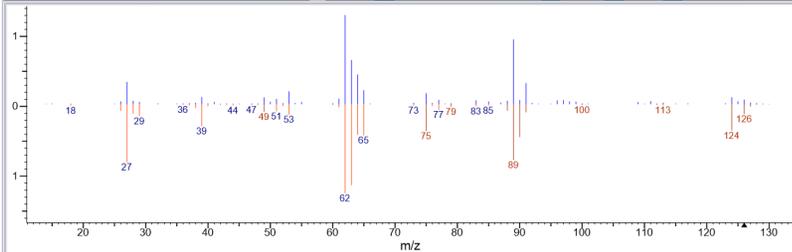
	Action	Result
7	Click <b>(i)</b> in the <b><math>\Delta m</math> Info</b> column. This brings up the <b>Adaptive Corrections</b> html page which explains how selective peaks have been shifted to obtain good <b>Hit Quality Index (HQI)</b> .	 <p>The following correction was applied to the reference spectrum to get optimal results:</p> <p><b>Some peaks in the reference spectrum were offset horizontally by 1 u</b></p> <p>The top spectrum is the unknown spectrum, the bottom spectrum is the unshifted reference spectrum, and the middle one is the shifted spectrum. In the shifted spectrum, the darker red peaks are additions of unshifted peaks and shifted peaks. Dotted lines between shifted and reference spectrum link shifted peaks to their original positions.</p>

Action	Result
8	<ul style="list-style-type: none"> <li>• The top spectrum is the unknown</li> <li>• The bottom spectrum is the unshifted reference</li> <li>• The middle "spectrum" contains shifted peaks</li> <li>• Dotted lines indicate what peaks have been moved by an <math>\Delta m</math></li> <li>• In the middle "spectrum," darker red peaks are the additions of existing peaks and moved ones</li> </ul>

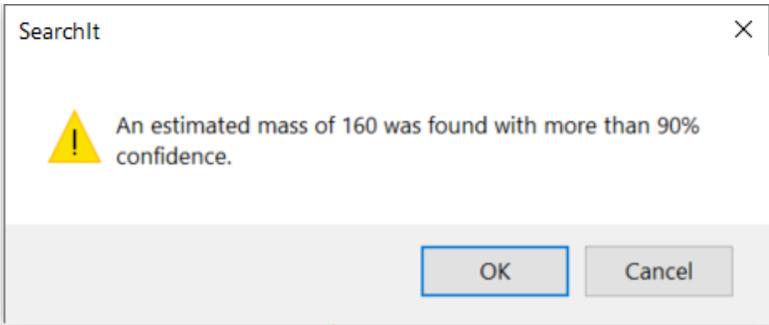
## Example wherein exact mass is NOT in a spectrum

	Action	Result
1	<p>Start a new search by clicking .</p> <p>Click <b>Spectrum</b> and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS folder.</p> <p>Select file <b>1,1,1-Trichlorobutane</b>.</p> <p>Use the drop-down menu to set the <b>Number of components</b> to <b>1 (Single)</b>.</p> <p>Ensure <b>Adaptive Search</b> or <b>Reverse Search</b> is unchecked.</p> <p>Click <b>User-Select</b>.</p> <p>Use <b>Remove All</b> to clean selected databases.</p> <p>Use <b>Add</b> to add MS (GC) databases by codes: <b>WMS3X</b>.</p> <p>Click <b>Search</b>.</p>	 <p>As we can see, this spectrum does not contain molecular ion mass. KnowItAll will estimate this value and use it for <b>Adaptive Search</b>.</p>

Action	Result																																																												
<p>2 Click the <b>Butterfly view</b> icon .</p> <p>Go to the 2<sup>nd</sup> hit since the 1<sup>st</sup> hit is the exact match (pretend that we do not have the exact match in our databases).</p>	 <div style="display: flex; justify-content: space-between;"> <div data-bbox="856 576 1648 820"> <p>MS (GC)</p> <table border="1"> <thead> <tr> <th>Table</th> <th>Plot</th> <th colspan="3">Related Compounds View</th> <th>Spectrum</th> </tr> <tr> <th>HQI</th> <th>R.HQI</th> <th>Tag</th> <th>ID</th> <th>Name</th> <th>&lt;auto&gt; (MS (GC))</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>100.00</td> <td>100.00</td> <td>VMS3_56884</td> <td>1,1,1-Trichlorobutane</td> <td></td> </tr> <tr style="background-color: #e0f0ff;"> <td>2</td> <td>78.93</td> <td>92.51</td> <td>VMS3_16290</td> <td>Cyclobutane, 1,2-dichloro-</td> <td></td> </tr> <tr> <td>3</td> <td>64.80</td> <td>65.18</td> <td>VMS3_16286</td> <td>2-Butene, 1,4-dichloro-</td> <td></td> </tr> <tr> <td>4</td> <td>64.23</td> <td>64.59</td> <td>VMS3_16299</td> <td>1-Butene, 3,4-dichloro-</td> <td></td> </tr> </tbody> </table> </div> <div data-bbox="1648 324 1948 820"> <p>Structure/Properties</p>  <table border="1"> <thead> <tr> <th>Name</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td>Cyclobutane, 1,2-dichloro-</td> </tr> <tr> <td>Alternate Name(s)</td> <td>1,2-Dichlorocyclobutane 1,2-Bis(chloranyl)cyclobutane</td> </tr> <tr> <td>CAS Registry Number</td> <td>17437-39-7</td> </tr> <tr> <td>Classification</td> <td>Organochlorides</td> </tr> <tr> <td>Estimated Kovats Retention Index</td> <td>813</td> </tr> <tr> <td>Exact Mass</td> <td>123.984656 u</td> </tr> <tr> <td>Formula</td> <td>C4H6Cl2</td> </tr> <tr> <td>InChI</td> <td>InChI=1S/C4H6Cl2/c5-3-1-2-4...</td> </tr> <tr> <td>InChIKey</td> <td>MPWHMPULOPKVVDQ-UHFFFA...</td> </tr> <tr> <td>Molecular Weight</td> <td>124.996 g/mol</td> </tr> <tr> <td>Nominal Mass</td> <td>124 u</td> </tr> </tbody> </table> </div> </div> <p>We are not sure by looking at the HQI that we have a hit that is structurally similar to our unknown.</p>	Table	Plot	Related Compounds View			Spectrum	HQI	R.HQI	Tag	ID	Name	<auto> (MS (GC))	1	100.00	100.00	VMS3_56884	1,1,1-Trichlorobutane		2	78.93	92.51	VMS3_16290	Cyclobutane, 1,2-dichloro-		3	64.80	65.18	VMS3_16286	2-Butene, 1,4-dichloro-		4	64.23	64.59	VMS3_16299	1-Butene, 3,4-dichloro-		Name	Value	Name	Cyclobutane, 1,2-dichloro-	Alternate Name(s)	1,2-Dichlorocyclobutane 1,2-Bis(chloranyl)cyclobutane	CAS Registry Number	17437-39-7	Classification	Organochlorides	Estimated Kovats Retention Index	813	Exact Mass	123.984656 u	Formula	C4H6Cl2	InChI	InChI=1S/C4H6Cl2/c5-3-1-2-4...	InChIKey	MPWHMPULOPKVVDQ-UHFFFA...	Molecular Weight	124.996 g/mol	Nominal Mass	124 u
Table	Plot	Related Compounds View			Spectrum																																																								
HQI	R.HQI	Tag	ID	Name	<auto> (MS (GC))																																																								
1	100.00	100.00	VMS3_56884	1,1,1-Trichlorobutane																																																									
2	78.93	92.51	VMS3_16290	Cyclobutane, 1,2-dichloro-																																																									
3	64.80	65.18	VMS3_16286	2-Butene, 1,4-dichloro-																																																									
4	64.23	64.59	VMS3_16299	1-Butene, 3,4-dichloro-																																																									
Name	Value																																																												
Name	Cyclobutane, 1,2-dichloro-																																																												
Alternate Name(s)	1,2-Dichlorocyclobutane 1,2-Bis(chloranyl)cyclobutane																																																												
CAS Registry Number	17437-39-7																																																												
Classification	Organochlorides																																																												
Estimated Kovats Retention Index	813																																																												
Exact Mass	123.984656 u																																																												
Formula	C4H6Cl2																																																												
InChI	InChI=1S/C4H6Cl2/c5-3-1-2-4...																																																												
InChIKey	MPWHMPULOPKVVDQ-UHFFFA...																																																												
Molecular Weight	124.996 g/mol																																																												
Nominal Mass	124 u																																																												

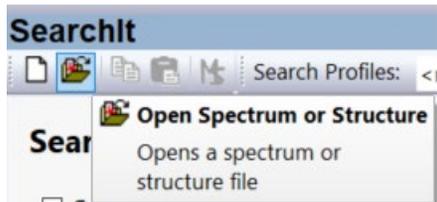
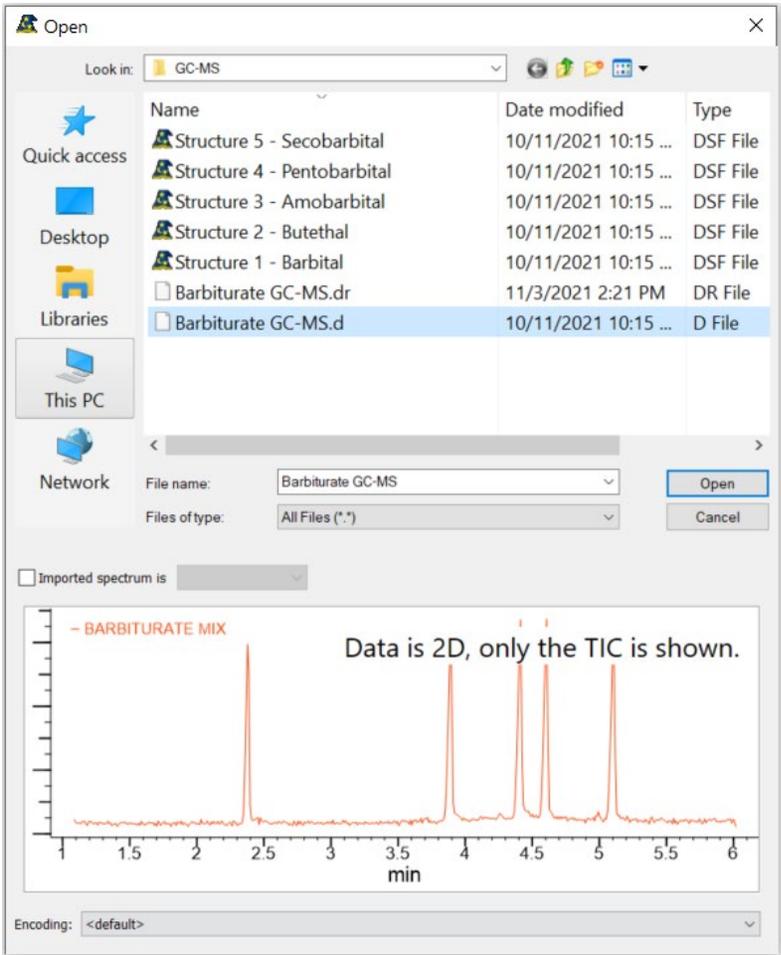
Action	Result																																																			
<p>3 Go back to <b>SearchIt</b></p> <p>Check <b>Adaptive Search</b></p> <p><input checked="" type="checkbox"/> Adaptive Search Molecular <i>m/z</i>: <input type="text"/></p> <p><b>Search</b></p> <p>In <b>Minelt</b>, Click the <b>Butterfly view icon</b> .</p> <p>Go to the 2<sup>nd</sup> hit (1<sup>st</sup> being the unknown which is already in a database).</p>	 <table border="1" data-bbox="856 576 1648 812"> <thead> <tr> <th>Table</th> <th>Plot</th> <th>Related Compounds View</th> </tr> <tr> <th>HQI</th> <th>R.H.C</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>&lt;auto&gt; (MS (GC))</th> <th>Δm [u]</th> <th>Δm Info</th> <th>Replacement</th> </tr> </thead> <tbody> <tr> <td>100.00</td> <td>100.00</td> <td>VMS3</td> <td>56884</td> <td>1,1,1-Trichlorobutane</td> <td></td> <td></td> <td>0</td> <td></td> <td></td> </tr> <tr> <td>82.79</td> <td>67.26</td> <td>VMS3</td> <td>17731</td> <td>Butane, 2,3-dichloro-</td> <td></td> <td></td> <td>34</td> <td>In unknown, Chlorine replaces Hydrogen in reference In unknown, -CF3 group replaces Chlorine in reference</td> <td></td> </tr> </tbody> </table> <div data-bbox="1648 324 1942 812"> <p>Structure/Properties</p>  <table border="1"> <thead> <tr> <th>Substructs</th> <th>Sel. Substructs</th> <th>Original Data Files</th> </tr> <tr> <th>All Properties</th> <th>Attachments</th> <th>Preferred Properties</th> </tr> <tr> <th>Name</th> <th colspan="2">Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td colspan="2">Butane, 2,3-dichloro-</td> </tr> <tr> <td>Alternate Name(s)</td> <td colspan="2">2,3-Dichlorobutane (-+/-)-2,3-Dichlorobutane 2,3-Bis(chloranyl)butane Butane, 2,3-dichloro-, (-+/-)- Butane, 2,3-dichloro-, (R*,S*)- Butane, 2,3-dichloro-, (R*,R*)-(+)- Butane, 2,3-dichloro-, (R*,R*)-(+/-)- Butane, 2,3-dichloro-, meso- dl-2,3-Dichlorobutane meso-2,3-Dichlorobutane racemic-2,3-Dichlorobutane EINECS 231-486-4</td> </tr> <tr> <td>CAS Registry Number</td> <td colspan="2">7581-97-7</td> </tr> </tbody> </table> </div> <p>One of the suggestions in the <b>Replacement</b> cell makes sense. The unknown should have 1 more Chlorine for a delta mass of 34.</p>	Table	Plot	Related Compounds View	HQI	R.H.C	DB	ID	Name	Spectrum	<auto> (MS (GC))	Δm [u]	Δm Info	Replacement	100.00	100.00	VMS3	56884	1,1,1-Trichlorobutane			0			82.79	67.26	VMS3	17731	Butane, 2,3-dichloro-			34	In unknown, Chlorine replaces Hydrogen in reference In unknown, -CF3 group replaces Chlorine in reference		Substructs	Sel. Substructs	Original Data Files	All Properties	Attachments	Preferred Properties	Name	Value		Name	Butane, 2,3-dichloro-		Alternate Name(s)	2,3-Dichlorobutane (-+/-)-2,3-Dichlorobutane 2,3-Bis(chloranyl)butane Butane, 2,3-dichloro-, (-+/-)- Butane, 2,3-dichloro-, (R*,S*)- Butane, 2,3-dichloro-, (R*,R*)-(+)- Butane, 2,3-dichloro-, (R*,R*)-(+/-)- Butane, 2,3-dichloro-, meso- dl-2,3-Dichlorobutane meso-2,3-Dichlorobutane racemic-2,3-Dichlorobutane EINECS 231-486-4		CAS Registry Number	7581-97-7	
Table	Plot	Related Compounds View																																																		
HQI	R.H.C	DB	ID	Name	Spectrum	<auto> (MS (GC))	Δm [u]	Δm Info	Replacement																																											
100.00	100.00	VMS3	56884	1,1,1-Trichlorobutane			0																																													
82.79	67.26	VMS3	17731	Butane, 2,3-dichloro-			34	In unknown, Chlorine replaces Hydrogen in reference In unknown, -CF3 group replaces Chlorine in reference																																												
Substructs	Sel. Substructs	Original Data Files																																																		
All Properties	Attachments	Preferred Properties																																																		
Name	Value																																																			
Name	Butane, 2,3-dichloro-																																																			
Alternate Name(s)	2,3-Dichlorobutane (-+/-)-2,3-Dichlorobutane 2,3-Bis(chloranyl)butane Butane, 2,3-dichloro-, (-+/-)- Butane, 2,3-dichloro-, (R*,S*)- Butane, 2,3-dichloro-, (R*,R*)-(+)- Butane, 2,3-dichloro-, (R*,R*)-(+/-)- Butane, 2,3-dichloro-, meso- dl-2,3-Dichlorobutane meso-2,3-Dichlorobutane racemic-2,3-Dichlorobutane EINECS 231-486-4																																																			
CAS Registry Number	7581-97-7																																																			

Alternatively, one can use the molecular ion mass estimation icon  to estimate the molecular ion mass:

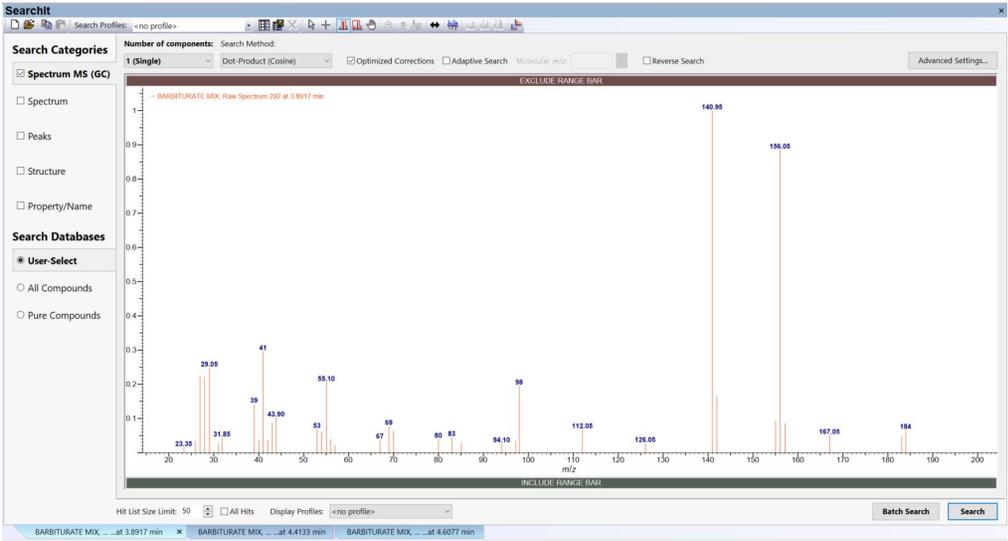
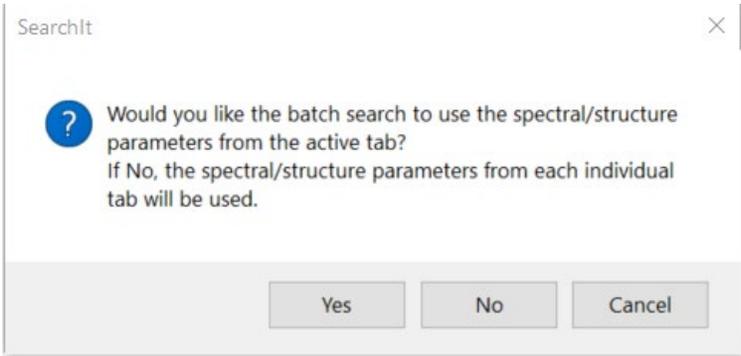
	Action	Result
1	Go Back to <b>SearchIt</b> .  Click the  icon  Let the calculation complete	
3	Click <b>OK</b> button	The estimated molecular ion mass value fills the box 

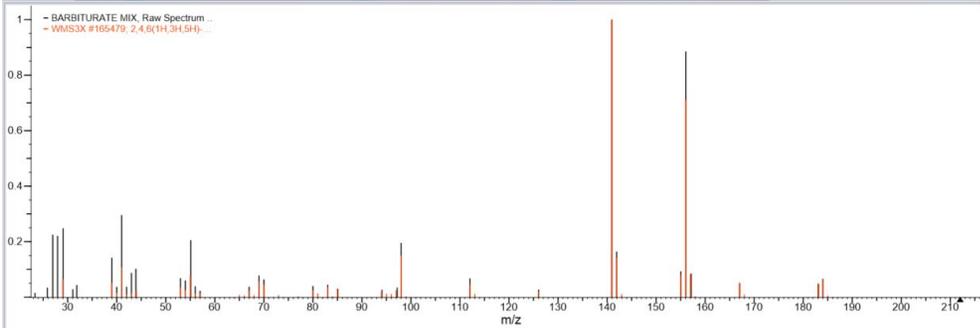
**NOTE:** KnowItAll runs an adaptive search of the unknown against all of our MS databases. The resulting hit list is then analyzed in steps. The mass of every hit list entry is calculated as the nominal mass of the compound in the database record plus the  $\Delta m$  found for the match. Matches of equal mass are then grouped together into clusters. The higher the found HQI, the higher the score for an individual match. Scores for clusters are then calculated as a combination of individual match scores with additional information such as the number of entries in the cluster and the separation from the next best cluster. The cluster with the best score determines the found mass. As side information of this procedure, information on confidence that the found mass will be correct is reported. The confidence values found by the algorithm were determined by running statistics with thousands of very diverse compound spectra run against our MS data.

## Simultaneous Multiple MS Spectra Search

Action	Result																								
<p>1 In <b>SearchIt</b>, select <b>Open Spectrum or Structure</b></p>  <p>Navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\GC-MS\Barbiturate GC-MS.d</p> <p>Click <b>Open</b> in previous dialog</p>	 <table border="1"> <thead> <tr> <th>Name</th> <th>Date modified</th> <th>Type</th> </tr> </thead> <tbody> <tr> <td>Structure 5 - Secobarbital</td> <td>10/11/2021 10:15 ...</td> <td>DSF File</td> </tr> <tr> <td>Structure 4 - Pentobarbital</td> <td>10/11/2021 10:15 ...</td> <td>DSF File</td> </tr> <tr> <td>Structure 3 - Amobarbital</td> <td>10/11/2021 10:15 ...</td> <td>DSF File</td> </tr> <tr> <td>Structure 2 - Butethal</td> <td>10/11/2021 10:15 ...</td> <td>DSF File</td> </tr> <tr> <td>Structure 1 - Barbital</td> <td>10/11/2021 10:15 ...</td> <td>DSF File</td> </tr> <tr> <td>Barbiturate GC-MS.dr</td> <td>11/3/2021 2:21 PM</td> <td>DR File</td> </tr> <tr> <td>Barbiturate GC-MS.d</td> <td>10/11/2021 10:15 ...</td> <td>D File</td> </tr> </tbody> </table> <p>File name: Barbiturate GC-MS</p> <p>Files of type: All Files (*.*)</p> <p>Imported spectrum is: [dropdown]</p> <p>Chromatogram: BARBITURATE MIX. Data is 2D, only the TIC is shown. X-axis: min (1 to 6).</p> <p>Encoding: &lt;default&gt;</p>	Name	Date modified	Type	Structure 5 - Secobarbital	10/11/2021 10:15 ...	DSF File	Structure 4 - Pentobarbital	10/11/2021 10:15 ...	DSF File	Structure 3 - Amobarbital	10/11/2021 10:15 ...	DSF File	Structure 2 - Butethal	10/11/2021 10:15 ...	DSF File	Structure 1 - Barbital	10/11/2021 10:15 ...	DSF File	Barbiturate GC-MS.dr	11/3/2021 2:21 PM	DR File	Barbiturate GC-MS.d	10/11/2021 10:15 ...	D File
Name	Date modified	Type																							
Structure 5 - Secobarbital	10/11/2021 10:15 ...	DSF File																							
Structure 4 - Pentobarbital	10/11/2021 10:15 ...	DSF File																							
Structure 3 - Amobarbital	10/11/2021 10:15 ...	DSF File																							
Structure 2 - Butethal	10/11/2021 10:15 ...	DSF File																							
Structure 1 - Barbital	10/11/2021 10:15 ...	DSF File																							
Barbiturate GC-MS.dr	11/3/2021 2:21 PM	DR File																							
Barbiturate GC-MS.d	10/11/2021 10:15 ...	D File																							

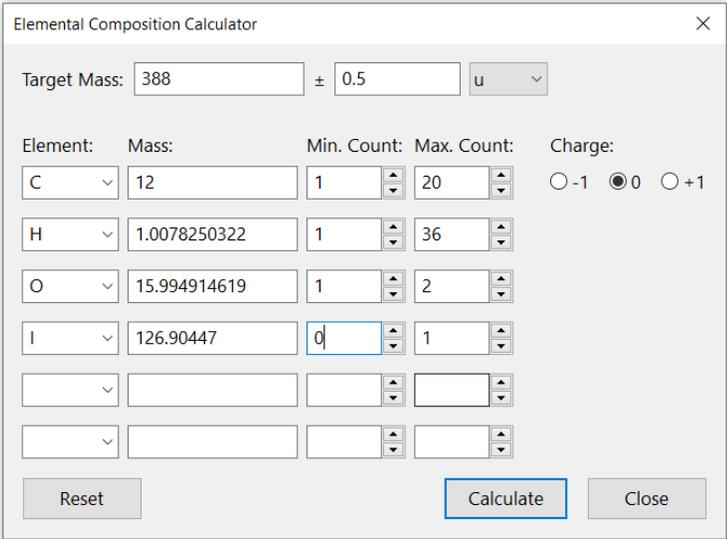
	Action	Result
2	<p>Pick multiple MS spectrum in the <b>MS Spectra Scan Selection</b> dialog</p> <p>For example, 292, 346, 366</p> <p>Click <b>OK</b> button</p>	<p> <input type="checkbox"/> 349 4.444  <input type="checkbox"/> 350 4.45333  <input type="checkbox"/> 351 4.46317  <input type="checkbox"/> 352 4.4725  <input type="checkbox"/> 353 4.48233  <input type="checkbox"/> 354 4.49167  <input type="checkbox"/> 355 4.50133  <input type="checkbox"/> 356 4.51083  <input type="checkbox"/> 357 4.5205  <input type="checkbox"/> 358 4.53  <input type="checkbox"/> 359 4.53967  <input type="checkbox"/> 360 4.54917  <input type="checkbox"/> 361 4.55883  <input type="checkbox"/> 362 4.56917  <input type="checkbox"/> 363 4.579  <input type="checkbox"/> 364 4.58833  <input type="checkbox"/> 365 4.59817  <input checked="" type="checkbox"/> 366 4.60767  <input type="checkbox"/> 367 4.61733  <input type="checkbox"/> 368 4.62767         </p> <p> <input type="button" value="Select All"/> <input type="button" value="Deselect All"/> <span style="margin-left: 20px;">Time of current scan: 2.136 min</span> </p> <p>             Spectrum import mode  <input checked="" type="checkbox"/> Import MS spectrum/spectra  <input type="checkbox"/> Import chromatogram         </p> <p style="text-align: right;"> <input type="button" value="OK"/> <input type="button" value="Cancel"/> </p>

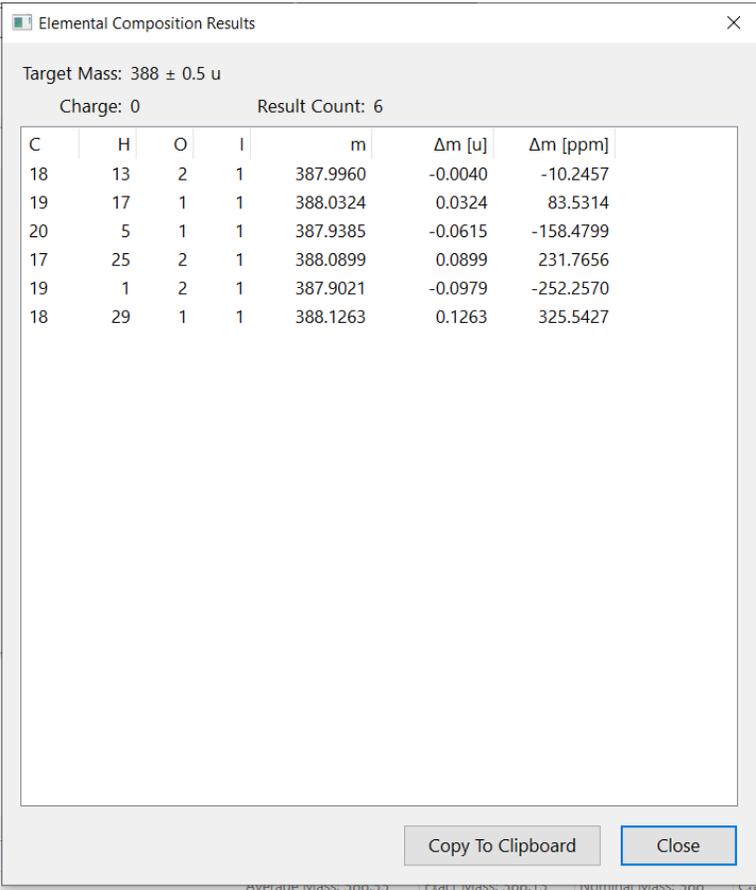
Action	Result
<p>3 Multiple search tabs are created</p>	
<p>4 One can either search these MS spectra one by one by clicking the <b>Search</b> button in each tab</p> <p>Or use <b>Do Batch Search</b> button and reply <b>Yes</b> when prompted</p>	
<p>5</p>	<p>Multiple hits are transferred to <b>Minelt</b> if <b>Do Batch Search</b> was selected</p>

Action	Result																																																																					
	<div data-bbox="863 305 1843 1068"> <p><b>Minelit</b></p> <p>Display Profiles: &lt;no profile&gt;</p> <p>Lookup Compound: PubChem</p>  <p>MS (GC)</p> <table border="1"> <thead> <tr> <th>Table</th> <th>Plot</th> <th>Related Compounds View</th> </tr> <tr> <th>HQI</th> <th>R.HQI</th> <th>Tag</th> <th>DF</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>&lt;auto&gt; (MS (GC))</th> <th>Δm (u)</th> <th>Δm Info</th> <th>Replacement</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>91.90</td> <td>95.75</td> <td>/MS3</td> <td>165479</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-</td> <td></td> <td>&lt;auto&gt;</td> <td>0</td> <td>ⓘ</td> <td></td> </tr> <tr> <td>2</td> <td>88.47</td> <td>89.62</td> <td>/MS3</td> <td>165488</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-</td> <td></td> <td>&lt;auto&gt;</td> <td>0</td> <td>ⓘ</td> <td></td> </tr> <tr> <td>3</td> <td>88.42</td> <td>91.28</td> <td>/MS3</td> <td>101117</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-</td> <td></td> <td>&lt;auto&gt;</td> <td>89</td> <td>ⓘ</td> <td></td> </tr> <tr> <td>4</td> <td>87.26</td> <td>84.84</td> <td>/MS3</td> <td>201919</td> <td>Pentobarbital</td> <td></td> <td>&lt;auto&gt;</td> <td>-14</td> <td>ⓘ</td> <td>In unknown, CH2 group replaces C=O group in reference. In unknown, Methyl group</td> </tr> <tr> <td>5</td> <td>87.08</td> <td>17.86</td> <td>/MS3</td> <td>238804</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-3-ethyl-1,3-2,4,6(1H,3H,5H)-</td> <td></td> <td>&lt;auto&gt;</td> <td>-28</td> <td>ⓘ</td> <td>In unknown, CH2 group replaces</td> </tr> </tbody> </table> <p>Hit List: BARBITURATE MIX, Raw Spectrum 345 at 4.4038 min    Hit List: BARBITURATE MIX, Raw Spectrum 366 at 4.6077 min    Hit List: BARBITURATE MIX, Raw Spectrum 292 at 3.8917 m</p> <p>1    200 Records</p> </div>	Table	Plot	Related Compounds View	HQI	R.HQI	Tag	DF	ID	Name	Spectrum	<auto> (MS (GC))	Δm (u)	Δm Info	Replacement	1	91.90	95.75	/MS3	165479	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-		<auto>	0	ⓘ		2	88.47	89.62	/MS3	165488	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-		<auto>	0	ⓘ		3	88.42	91.28	/MS3	101117	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-		<auto>	89	ⓘ		4	87.26	84.84	/MS3	201919	Pentobarbital		<auto>	-14	ⓘ	In unknown, CH2 group replaces C=O group in reference. In unknown, Methyl group	5	87.08	17.86	/MS3	238804	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-3-ethyl-1,3-2,4,6(1H,3H,5H)-		<auto>	-28	ⓘ	In unknown, CH2 group replaces
Table	Plot	Related Compounds View																																																																				
HQI	R.HQI	Tag	DF	ID	Name	Spectrum	<auto> (MS (GC))	Δm (u)	Δm Info	Replacement																																																												
1	91.90	95.75	/MS3	165479	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-		<auto>	0	ⓘ																																																													
2	88.47	89.62	/MS3	165488	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-		<auto>	0	ⓘ																																																													
3	88.42	91.28	/MS3	101117	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-		<auto>	89	ⓘ																																																													
4	87.26	84.84	/MS3	201919	Pentobarbital		<auto>	-14	ⓘ	In unknown, CH2 group replaces C=O group in reference. In unknown, Methyl group																																																												
5	87.08	17.86	/MS3	238804	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-3-ethyl-1,3-2,4,6(1H,3H,5H)-		<auto>	-28	ⓘ	In unknown, CH2 group replaces																																																												

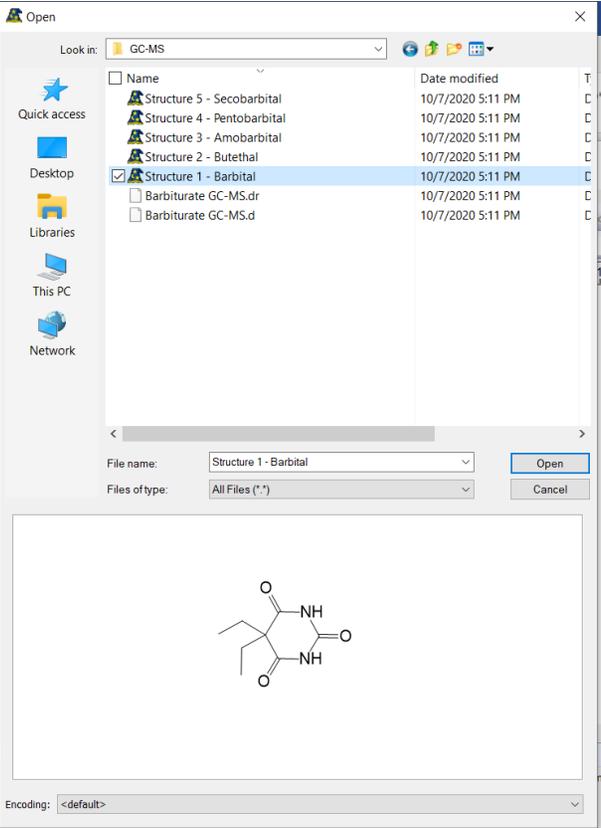
## Other Tools for Mass Spectrometry

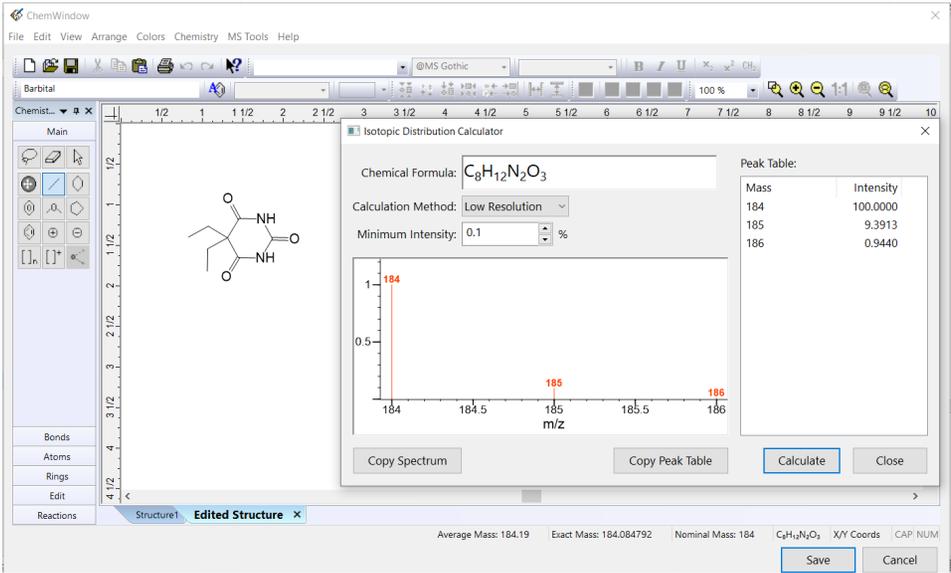
### Elemental Composition

	Action	Result
1	From the <b>Menu</b> , navigate to <b>MS Tools &gt; Calculate Elemental Composition</b> .	 <p data-bbox="642 1068 1369 1097">You can fill in this dialog with target mass and elemental information.</p>

	Action	Result																																																	
2	Click <b>Calculate</b> .	 <p>Elemental Composition Results</p> <p>Target Mass: 388 ± 0.5 u</p> <p>Charge: 0      Result Count: 6</p> <table border="1"><thead><tr><th>C</th><th>H</th><th>O</th><th>I</th><th>m</th><th>Δm [u]</th><th>Δm [ppm]</th></tr></thead><tbody><tr><td>18</td><td>13</td><td>2</td><td>1</td><td>387.9960</td><td>-0.0040</td><td>-10.2457</td></tr><tr><td>19</td><td>17</td><td>1</td><td>1</td><td>388.0324</td><td>0.0324</td><td>83.5314</td></tr><tr><td>20</td><td>5</td><td>1</td><td>1</td><td>387.9385</td><td>-0.0615</td><td>-158.4799</td></tr><tr><td>17</td><td>25</td><td>2</td><td>1</td><td>388.0899</td><td>0.0899</td><td>231.7656</td></tr><tr><td>19</td><td>1</td><td>2</td><td>1</td><td>387.9021</td><td>-0.0979</td><td>-252.2570</td></tr><tr><td>18</td><td>29</td><td>1</td><td>1</td><td>388.1263</td><td>0.1263</td><td>325.5427</td></tr></tbody></table> <p>Copy To Clipboard      Close</p> <p>KnowItAll provides combinations of these elements.</p>	C	H	O	I	m	Δm [u]	Δm [ppm]	18	13	2	1	387.9960	-0.0040	-10.2457	19	17	1	1	388.0324	0.0324	83.5314	20	5	1	1	387.9385	-0.0615	-158.4799	17	25	2	1	388.0899	0.0899	231.7656	19	1	2	1	387.9021	-0.0979	-252.2570	18	29	1	1	388.1263	0.1263	325.5427
C	H	O	I	m	Δm [u]	Δm [ppm]																																													
18	13	2	1	387.9960	-0.0040	-10.2457																																													
19	17	1	1	388.0324	0.0324	83.5314																																													
20	5	1	1	387.9385	-0.0615	-158.4799																																													
17	25	2	1	388.0899	0.0899	231.7656																																													
19	1	2	1	387.9021	-0.0979	-252.2570																																													
18	29	1	1	388.1263	0.1263	325.5427																																													

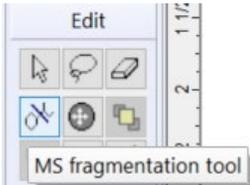
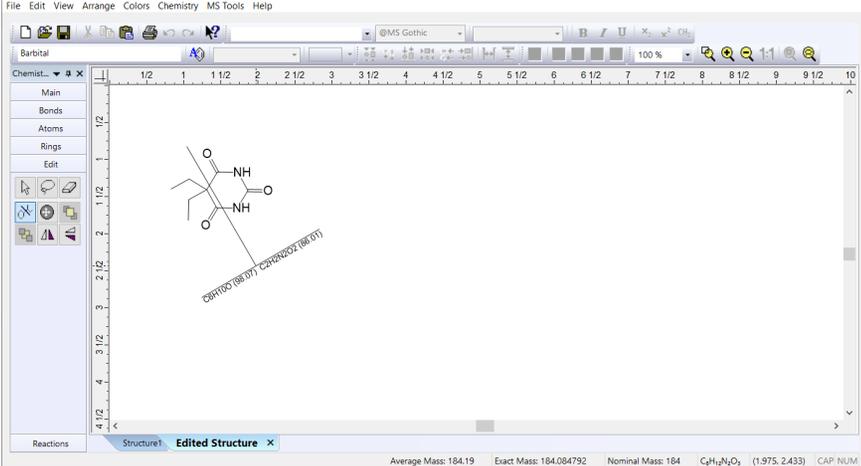
## Isotopic Distribution

	Action	Result																
1	<p>Double-click the structure pane to bring up ChemWindow or use the Transfer to: bar and select <b>ChemWindow</b> at the top of the application.</p> <p>Click <b>File &gt; Open</b>.</p> <p>Select a structure from the <b>Samples &gt; GC-MS</b> folder.</p>	 <p>The screenshot shows a Windows File Explorer window titled 'Open' with the 'Look in:' path set to 'GC-MS'. The file list contains the following entries:</p> <table border="1"><thead><tr><th>Name</th><th>Date modified</th></tr></thead><tbody><tr><td>Structure 5 - Secobarbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td>Structure 4 - Pentobarbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td>Structure 3 - Amobarbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td>Structure 2 - Butethal</td><td>10/7/2020 5:11 PM</td></tr><tr><td><input checked="" type="checkbox"/> Structure 1 - Barbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td><input type="checkbox"/> Barbiturate GC-MS.dr</td><td>10/7/2020 5:11 PM</td></tr><tr><td><input type="checkbox"/> Barbiturate GC-MS.d</td><td>10/7/2020 5:11 PM</td></tr></tbody></table> <p>The 'File name:' field contains 'Structure 1 - Barbital' and the 'Files of type:' dropdown is set to 'All Files (*.*)'. The chemical structure of Barbital is shown below the file list.</p> <chem>CC(C)C(=O)NC(=O)C1(C)C(=O)NC1=O</chem>	Name	Date modified	Structure 5 - Secobarbital	10/7/2020 5:11 PM	Structure 4 - Pentobarbital	10/7/2020 5:11 PM	Structure 3 - Amobarbital	10/7/2020 5:11 PM	Structure 2 - Butethal	10/7/2020 5:11 PM	<input checked="" type="checkbox"/> Structure 1 - Barbital	10/7/2020 5:11 PM	<input type="checkbox"/> Barbiturate GC-MS.dr	10/7/2020 5:11 PM	<input type="checkbox"/> Barbiturate GC-MS.d	10/7/2020 5:11 PM
Name	Date modified																	
Structure 5 - Secobarbital	10/7/2020 5:11 PM																	
Structure 4 - Pentobarbital	10/7/2020 5:11 PM																	
Structure 3 - Amobarbital	10/7/2020 5:11 PM																	
Structure 2 - Butethal	10/7/2020 5:11 PM																	
<input checked="" type="checkbox"/> Structure 1 - Barbital	10/7/2020 5:11 PM																	
<input type="checkbox"/> Barbiturate GC-MS.dr	10/7/2020 5:11 PM																	
<input type="checkbox"/> Barbiturate GC-MS.d	10/7/2020 5:11 PM																	

	Action	Result								
2	<p>Navigate to <b>MS Tools &gt; Calculate Isotopic Distribution</b>.</p> <p>Click <b>Calculate</b>.</p>	 <p>The screenshot shows the ChemWindow interface with the Isotopic Distribution Calculator dialog box open. The chemical formula is <math>C_8H_{12}N_2O_3</math>. The calculation method is set to Low Resolution, and the minimum intensity is 0.1%. The resulting peak table is as follows:</p> <table border="1"> <thead> <tr> <th>Mass</th> <th>Intensity</th> </tr> </thead> <tbody> <tr> <td>184</td> <td>100.0000</td> </tr> <tr> <td>185</td> <td>9.3913</td> </tr> <tr> <td>186</td> <td>0.9440</td> </tr> </tbody> </table> <p>The mass spectrum plot shows peaks at m/z 184, 185, and 186. The peak at m/z 184 is the base peak with an intensity of 1.0. The peak at m/z 185 has an intensity of approximately 0.09, and the peak at m/z 186 has an intensity of approximately 0.01.</p>	Mass	Intensity	184	100.0000	185	9.3913	186	0.9440
Mass	Intensity									
184	100.0000									
185	9.3913									
186	0.9440									

## Molecular Fragmentation

In ChemWindow, you can use the MS fragmentation tools to view possible fragments and corresponding masses.

	Action	Result
1	<p>In the <b>Edit</b> toolbar on the left, click on the <b>MS fragmentation tool</b>.</p>  <p>Point to a position, then drag a line—this is the fragmentation line.</p>	 <p>Chemist... ▾ x</p> <p>Main Bonds Atoms Rings Edit</p> <p>Reactions</p> <p>Structure1 Edited Structure x</p> <p>Average Mass: 184.19 Exact Mass: 184.084792 Nominal Mass: 184 C<sub>4</sub>H<sub>4</sub>N<sub>3</sub>O<sub>3</sub> (1.975, 2.433) CAP NUM</p>

## Solid Triangle Marks Nominal Mass

- In the spectrum display, KnowItAll marks the nominal mass of the structure that corresponds to the spectrum. This mass is shown as a black triangle.
- When importing a spectrum from a data file, a number of import formats define fields for molecular  $m/z$  (also called precursor  $m/z$  or base peak  $m/z$  in some cases) and the charge of the molecular ion. To convert from molecular  $m/z$  to exact mass, the following formulae are used:
  - For positive charges:
    - $M_{\text{exact}} = (Mz - M(\text{H}) + M(\text{e})) * \text{charge}$ , where  $M(\text{H})$  is the mass of a hydrogen atom, and  $M(\text{e})$  is the mass of an electron.
  - For negative charges:
    - $M_{\text{exact}} = (Mz - M(\text{e})) * (-\text{charge})$ .
  - If no charge is defined, a default charge of +1 is assumed.
- If a data file does not have the molecular  $m/z$  field defined, the exact mass is calculated from the formula field, if available.